An individual based population study of an insect herbivore (*Urophora jaceana*) and its host plant (*Centaurea nigra*)

Thesis

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An individual based population study of an insect herbivore (*Urophora jaceana*) and its host plant (*Centaurea nigra*).

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Abstract *- Urophora jaceana* Hering (Diptera; Tephritidae) is a gall-forming insect of the capitula of *Centaurea nigra* L. (Compositae). Oviposition occurs at an early stage of flowerbud development. The aim of this thesis is to relate population processes of regulation of fly numbers and phenotypic selection on plant characters to aspects of individual behaviour of *Urophora jaceana*, through the development of a Dynamic State Variable Model of female fly oviposition choices.

The dynamic model incorporates larval survival functions obtained experimentally, and foraging time budgets estimated from field observations. The model predicts clutch size and super-oviposition at varying egg-loads, age, and host density. Super-oviposition is more likely at low encounter rates with flowerheads, and this prediction was tested experimentally and found to hold. Using a Monte Carlo Simulation, competition for oviposition sites, and the inverse dependence of larval survival on clutch size at high egg densities, emerge as the most likely regulating factors of fly numbers.

The effect of gall infestation on plants was found to be limited to reducing seed production. No density dependent regulation of fly density was discovered by k-factor analysis in the gall formation to pupal phase of the life cycle. The intensity of gall infestation was found to be inversely dependent on day of budding of flowerbuds, and relates to resource availability. Measuring the intensity of selection on three plant characters revealed that gall infestation exerts a directional selection pressure on the day of budding of plants. A simple heritability test suggests that there is an evolutionary response to selection.

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Part 1:
Introduction

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Chapter 1: Individuals and Populations

The guiding principle of this thesis is that population level phenomena are the outcome of interactions between individuals. It is an essentially reductionist view (Lomnicki 1988) which attempts to understand ecological processes from the detail of individual behaviour or phenotypic variation rather than from the assumption that an eco-system, community or population is an entity in itself, or as Lomnicki (1988) describes it, a super-organism.

Populations and communities are made up of individuals that differ among themselves, and unless individual variation is taken into account in ecological studies, crucial evidence concerning the dynamics of ecological systems will be missed (Lomnicki 1988). Begon (1984) pointed out "the ubiquitous concept of density disguises the real level at which organisms interact". Skewness and assymmetric competition are an inherent feature of many populations, masked often by the assumption of normal distribution of phenotypes (Begon 1984). Begon (1984) suggests that individual differences may therefore be important in the way the population is regulated, for example, in the study of life-history strategies and in predator prey dynamics. In fact, some population models assume individual differences (Hassell and May 1985), for example in the consideration of the spatial distribution of a population, which is ultimately determined by the foraging behaviour of individuals.

In considering population regulation, the debate continues as to whether the principle component is the effect of natural enemies which respond in a density dependent way to population changes, or whether it is limited resources, such as food or oviposition sites, which determine fluctuations in populations (Begon and Mortimer 1986, Chapter 7). Density dependant processes on the population scale are often difficult to establish (Hassell 1985, Dempster 1983, Dempster et al 1995a), and the availability of resources, especially in insect studies often seems to be abundant (Straw 1991). Considering individual differences can help here to understand some of the processes at work in regulating populations. For example, taking into account resource availability as it varies within a season, or between patches, suggests that resource limitation is an important factor in determining insect densities (Dempster et al 1995a, Dempster and Pollard 1981, Straw 1991). The variation in the timing of life history stages ensures that all resources are not available at once. Asynchronicity (Hassell 1994, Iwasa and Levin 1995) may be an important element of population regulation which also rests on individual differences.

Individual variation within a population will often have a genetic component (Bradshaw 1984). Ecological processes and interactions can therefore have consequences for natural selection within populations, if they give advantage to some phenotypes over others. Where there are ecologically significant individual differences and a high turnover of individuals in a population, evolution would be expected to be taking place (Bradshaw 1984). Methods for measuring the nature, direction and intensity of selection in natural populations have been developed (Lande

and Arnold 1983) which confirm that this is so, but also reveal a degree of complexity, such as the direction of selection varying between seasons (Haldane and Jayaker 1963).

Observations at the population level can sometimes be understood in terms of the decisions that individuals make (Krebs and Kacelnik 1991), for example, when a parasitoid oviposits in a host already containing eggs (super-parasitism), or when even in a resource limited environment predation is still the main cause of mortality. An essential part of the process of understanding populations and communities, therefore, is the development of models which link individual behaviour to population effects. A key concept here is that of optimal strategies of behaviour (Krebs and Kacelnik 1991), in which an individual will make decisions about its behaviour which will maximise its success, often expressed in terms of fitness or its components. A distinction needs to be made between an individual optimising present gains and optimising lifetime fitness (Krebs and Kacelnik 1991). Optimising lifetime fitness will involve trade-offs between present risks and future gains, and is a concept which can explain surprising behavioural decisions.

Some attempts have been made to develop models which work explicitly from individuals to population level. For example, in modelling populations of oyster-catcher (*Haematopus ostralegus*) at different densities, Goss Custard et al (1995a, 1995b, 1995c) applied game theory to the individual foraging decisions made by a model population. The choices made by individuals in the model, concerning where and how to search for food, affect food resources and the behaviour of other birds (Goss Custard et al (1995c). The purpose of this study was to predict the effects of the reduction of habitat on oyster-catcher populations.

Using detailed estimation of the parameters of individual foraging behaviour (Goss-Custard et al 1995b) predicting population effects of drastic reduction of habitat was made possible.

The dynamic state variable models of Mangel and Clark (1988) explicitly utilise the concept of optimising fitness, in which decisions made by individuals affect their state, enabling important variables such as food reserves, eggload, and age of the individual to be incorporated into the model. In addition the changes in the environment, such as the distribution of hosts, which are the result of individual decisions can also be included (Mangel and Clark 1988). Using either game theory, or the simpler, but more limited, Monte Carlo simulation technique, Mangel and Clark (1988) demonstrate how important predictions concerning population dynamics can be made with these models. The dynamic modelling and Monte Carlo simulation approach is used in this thesis to link individual behaviour to population level phenomena.

This wide-ranging thesis attempts to balance detailed field and experimental work with a synthetic vision of the dynamics of the interaction between *Urophora jaceana* and its host plant *Centaurea nigra*. The over-arching aim of the thesis is to show what choices drive the individual behaviour of the fly, and how this behaviour affects the phenotypic distribution of the plant. In considering an interaction in which timing of oviposition, as for many species of tephritid flies (Straw 1989b, 1989c), is so crucial, and in which the distribution of available flowerbud hosts through time is so variable, the question is, do the phenotypic differences between plants play a part in the regulation of the insect population, and does the insect population impinge on the phenological distribution of the plant?

Chapter 2: Organisms, Field Study Site and Methods

Section 1: Organisms: *Centaurea nigra* L. (Compositae)

Centaurea nigra is found throughout Europe, and in most of the British Isles up to 400m altitude. It occurs in ungrazed limestone grassland, rock outcrops, pastures, meadows, road verges, but not in wetlands or woodlands. It is generally found on moderately fertile or infertile soils. Vesicular-arbuscular mycorrhizal fungi, which invade the cortical cells of the root to form sacs (vesicles) and branching structures (arbuscules), increase seedling yield up to eight-fold (Grime, Hodgson and Hunt 1988).

2.1.1 Taxonomy

Linnaeus identified fifty species in the genus *Centaurea*, including *Centaurea nigra*, and *Centaurea jacea*. Along with *Centaurea nemoralis* Jordan, these species form a very complex group, and it is unclear whether they should be classed as separate species or included under one species (Turril and Marsden Jones 1954; Lack 1976 and 1982; Elkington and Middlefell 1972).

Turril and Marsden Jones (1954) list the characters distinguishing *Centaurea nigra* and *Centaurea jacea*, and Elkington and Middlefell (1972) list those distinguishing *Centaurea nigra* and *Centaurea nemoralis*. It is very difficult to find individual plants which display all the characters of one or other of the species. It has been suggested (Turril and Marsden Jones 1954) that the blurred boundaries between the species

are due to hybridisation, which occurs readily. Alternatively it has been suggested (Ockendon, Walters and Whiffen 1969; Elkington and Middlefell 1972) that there is a steady cline between *Centaurea nigra* and *Centaurea nemoralis* and that a clear distinction cannot be made between them. Although the taxonomy of this group needs to be revised, it is usual to speak of the *Centaurea nigra* complex as comprising the two subspecies *nigra* and *nemoralis*, with *Centaurea jacea* a separate species.

The differences between *Centaurea nigra* and *Centaurea nemoralis* include distribution and flowering time (Lack 1976). *Centaurea nemoralis* occurs mainly in southern England, on chalk and limestone whereas *Centaurea nigra* extends to the north of Scotland, and is characteristic of neutral and acid grassland in the South. *Centaurea nigra* flowers in June and July in south, whereas *Centaurea nemoralis* in July to early September. Following Lack (1976), in this thesis *Centaurea nigra* is used in the inclusive sense to refer to the whole complex.

2.1.2 Phenology

Centaurea nigra is a polycarpic perennial, ie it produces fruits annually usually from the second year after germination (own observation). It has a semi-rosette form. Stems are typically 0.5cm to 1.2cm in diameter at the base, tapering to 0.3cm to 0.4cm and are often branched bearing a number of capitula. Leaves are lanceolate. The roots are infected by mycorrhizal fungi (Grime, Hodgson and Hunt 1988).

A rosette of leaves on an individual plant may survive over winter. New shoots grow in May. In the field site described elsewhere in this chapter, flowerbuds appear in mid June and flowering takes place

between mid July and mid August. Seed is shed in August through to September, and some seed may remain in the inflorescence after the shoot dies back in the autumn. Shoots grow to between 20cm and 80cm.

Flowers have up to 100 purple floret tubes which are gathered into a capitula, and are hermaphrodite. They are insect pollinated (Lack 1976, 1982), and are generally self-incompatible (see Turril and Marsden Jones 1954). Up to 100 seeds may be produced in each capitulum. Germination takes place in either autumn or spring. A few seeds may survive for a number of years, but there is not a persistent seed bank (Grime, Hodgson and Hunt 1988).

2.1.3 Vegetative reproduction

Reproduction is mainly by means of seed, but vegetative regeneration is also known (Turril and Marsden Jones, 1954). From the thickened portion of the base of the stem, lateral shoots grow out for a distance of about 2.5cm. The shoots end in a rosette of leaves. After 2 or 3 years the lateral shoot rots away, leaving an independent plant. The central plant may then die back due to shading, leaving a fairy ring of clones.

2.1.4 Sexual reproduction

Seeds are dispersed in one-seeded fruits called cypselas (Turril and Marsden Jones 1954). Cypselas have a vestigial pappus of short bristles, which appears to be of little use in dispersal. Seeds are shaken or knocked from an inflorescence, by wind or passing animals, and fall within 10 to 20 cms of the plant. It is difficult to ascertain lifetime reproductive capacity, because of the long life of plants under

cultivation, and the many variables in the wild. Annual reproductive capacity was investigated by Turril and Marsden Jones (1954). Early flowering plants may produce a second lot of flowers in late summer.

There are significant differences of flowering periods between different stocks and different populations. Plants from the wild generally breed true to flowering period (Lack 1976). Crossing early with late, and late with early plants produces a family of plants with medium flowering period (Turril and Marsden Jones 1954), suggesting a genetic basis for flowering period. Weather also appears to influence flowering period.

Lack (1976, 1982) discovered large differences of peak flowering time between populations of *Centaurea nigra* and between individuals of the same population. There is much variation in flowering time on which selection can act, and Lack suggests (1982) that competition for pollinators is one selection pressure which may favour later flowering.

Pollination is brought about by nectar-seeking insects (Turril and Marsden Jones 1954). An insect visiting a capitulum during anthesis will investigate a number of florets successively. Each floret has only one ovule, needing only one pollen grain to allow for fertilisation. In trials conducted by Turril and Marsden Jones (1954), self-incompatibility was complete or near complete.

2.1.5 Mortality

The average life may be three or four flowering seasons in the wild (Turril and Marsden Jones 1954). Close competition with dense vegetation also reduces the number of shoots produced the following year.

Section 2: Organisms:
Urophora jaceana

The capitula of *Centaurea nigra* are host to a complex community of insects which have been described in detail by Varley (1937; 1947). The knapweed gall fly, *Urophora jaceana* Hering (Diptera; Tephritidae) is a univoltine gall-forming herbivore. The descriptions below are summaries from Varley (1947), and my own observations.

2.2.1 Taxonomy

Up to 1937 the knapweed gall fly was known as *Urophora solstitialis* (L.). The continental species of this name is a gall fly of thistles. Hering, in 1935, first described the gall fly of *Centaurea jacea* under the name *jaceana*.

2.2.2 Life history

Adult *Urophora jaceana* emerge in mid-June to mid-July from the galls in which they have lived as larvae and as pupae from the previous year. Males and females can be easily distinguished in that females have a long oviscape at the rear of the abdomen, whereas the male does not.

Females lay eggs in clutches in the developing capitula of *Centaurea nigra*, when buds are at an early stage of development. A female walks onto a flowerbud and turns around a number of times. It then probes with its ovipositor, which extends out of the oviscape sheathing it, into

the soft tissue at the base of bud, and curls the end of the ovipositor so that it reaches the space above the floret tubes inside the bud, where the eggs are laid (own observation).

The first instar of the larvae is spent inside the egg, and the second instar emerges after about 12 days (Varley 1947). The second instar larva burrows into a floret tube to the ovary, where gall tissue is rapidly induced. The tissue around the ovary rapidly expands to form a gall about 3mm by 7mm (Varley 1947). The passage through which the larva entered remains open, so that the gall cell is flask shaped. If more than one gall is produced within the flowerbud, they fuse together to form a gall complex containing a number of gall cells.

Three weeks after oviposition, the third instar emerges (Varley 1947). It continues to feed inside the gall cell, and turns its head to face the base of the cell. The hind end of the larva then hardens (becomes sclerotised) and pigmented (almost black), and is used to plug the open end of the cell, forming an effective protection against some natural enemies (Varley 1947). The third instar larva soon after is fully grown, during August or the beginning of September, and enters a dormant phase within the gall cell, where it over-winters. In May the following year, the larva turns round in the gall cell, so that its head is facing the opening, and its cuticle forms a hard brown puparium. It first enters a fourth instar, a pre-pupa, and then forms the pupa proper (Varley 1947). The adult emerges about a month later.

Plate 1: Adult

Urophora jaceana

1) Adult female

Magnified x12

2) Adult female

x12

3) Adult male

x12

2.2.3 Natural enemies: Parasitoids

The larvae and pupae of *Urophora jaceana* have natural enemies, of particular importance being chalcidoidae parasitoids. Table 1 lists those that have been identified in Britain. Of these, *Eurytoma curta*, *Torymus cyranimus*, *Tetrastichus sp. B*, and *Macroneura vesicularis*, have been identified in my study site.

Table 1: Chalcidoidae parasitoids attacking larvae and pupae of *Urophora jaceana*, after Varley (1947). Those which are asterixed have been identified in my study site.

Hymenoptera, Chalcidoidae	Ecto/Endo phagous	Gregarious/ solitary	Uni/multi voltine	Timing of attack
*Eurytoma curta**	Endo	Solitary	Uni	July
Eurytoma robusta	Ecto	Solitary	Uni	August
Habrocytus trypetae	Ecto	Solitary	Bi	May/August
*Torymus cyranimus**	Ecto	Solitary	Uni?	August
*Macroneura vesicularis**	Ecto	Gregarious	Bi	May/August
Tetrastichus brevicornus	Ecto	Gregarious	?	?
*Tetrastichus sp. B**	Endo	Gregarious	Bi	June/Aug
Apostocetus daira	Endo	Gregarious	?	?

Table 2.1: Key to Plate 2

1	Dissected flowerbud of *Centaurea nigra*, showing *Urophora jaceana* egg oviposited above the floret tubes, and traces of the pathway of the ovipositor of the gall fly (x15).
2	Second instar *Urophora jaceana* larva soon after gall formation. Note the head (the black point) facing upwards towards the mouth of the gall-cell (x50).
3	Second instar *Urophora jaceana* larva, having turned round in the gall-cell, with head pointing downwards. The darkened foot of the larva can be seen (x25).
4	Third instar *Urophora jaceana* larva, fully grown and filling the gall-cell (x25).
5	Pupa of *Urophora jaceana*, occupying gall-cell (x25).

Plate 2

1

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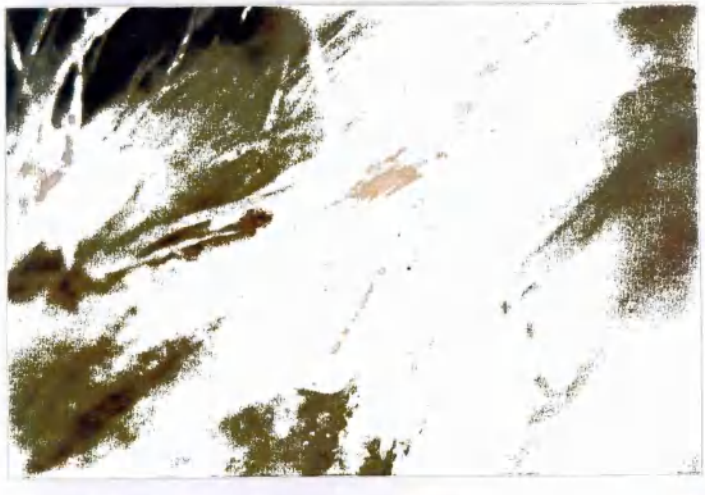

2

3

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Varley (1947) gives a full description of each of the parasitoids. The account given here summarises his main points.

Eurytoma curta attack the larvae of *Urophora jaceana* soon after they are hatched. Eggs of the parasitoid have only been found in second instar hosts, the protection afforded by their hardened rear end of the third instar making it impossible for eggs to be laid inside them. The larva is endophagous, and normally there is one larva per host. By the time the host larva has fully grown, the larva of *Eurytoma curta* is in its third instar. At this time, in late August, the parasitoid induces the host to turn round in its cell and to form the brown puparium, which normally happens in May. Inside the puparium the larva grows very rapidly, consuming its host in a few days, developing into a fifth instar larva, which over-winters inside the puparium shell. Adults emerge in the first half of July.

The larva of *Torymus cyranimus* is an ectoparasite of the larva of *Urophora jaceana*, and also of *Eurytoma curta*. Eggs are laid in August on the host larva. *Urophora jaceana* larvae parasitised by *Eurytoma curta* are not differentiated from those which are not parasitised. More than one oviposition in a single host (super-parasitism) may often occur. Growth is rapid, and normally only one larva survives in a single host. The winter is usually passed in the larval stage, and adults emerge in May of the following year. It is possible that a second generation develops in a different host species, or that adults live until suitable *Urophora jaceana* hosts are found in August.

Table 2.2: Key to Plate 3

1	Brown puparium of parasitised *Urophora jaceana* larva, containing a larva of *Eurytoma curta*.
2	Adult female *Eurytoma curta* (x11).
3	Larva of *Torymus cyanimus*, occupying a gall-cell (x25).
4	Adult *Torymus cyanimus* female (x11).
5	Adult *Macroneura vesicularis* female (x25).
6	Adult *Tatrastichus sp. B.* (x11).

Plate 3

2

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3

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Macroneura vesicularis is a polyphagous parasitoid, which appears to have two generations in the year. The early generation of adults parasitise the larvae or pupae of *Urophora jaceana* in May, one parasitoid larva developing in one host. The second generation emerges in August, and it is suggested by Varley (1947) that these adults find alternative hosts to *Urophora jaceana*. As with *Torymus cyranimus*, more than one oviposition (super-parasitism) may occur in a single host.

Tetrastichus sp. B is a gregarious endoparasitoid, with two generations in the year, adults emerging in June and August. In June, the previous year's galls are attacked, and in August the new galls, when the larvae persist over the winter in the skin of the host larva. Super-parasitism occurs commonly. Between three and twenty of this parasitoid may occupy one host.

2.2.4 Natural enemies: Lepidoptera

In addition to the Chalcidoidae parasitoids, the capitula insect community in my study area includes two species of lepidoptera (see Varley 1947 for full description): *Metzneria metzneriella* (Tynaioidiae), and *Eucosma hohenwartiana* (Tortricoidae). Eggs are laid in July and the larvae of both these species feed inside the capitula of *Centaurea nigra*, feeding on plant tissue, including gall tissue, and the contents of galls. On dissection, gall cells attacked by these larvae are empty apart from the frass left behind. This was found to be a significant cause of larval mortality of *Urophora jaceana* in my study site.

Section 3: The Field Study Site

The site used for field work between 1991 and 1996 is situated just outside the village of Warmington, South Warwickshire, UK, grid reference SP 3995 4740. The site is a large island between the two lanes of the B4100 where it divides into a dual carriageway on Warmington Hill. At its widest, it is 50m across, and at its longest, 150m.

The area has remained undisturbed for over twenty years, since the road was improved. Warwickshire County Council Department of Planning and Transportation are responsible for maintenance. A strip round the perimeter of the site, 3m wide, is mown annually in mid June, and the rest is left unmown. It is therefore an ideal site in which to study a plant insect interaction which requires survival of hard heads into the following year.

Permission to use the site was given in 1991 by the Department of Planning and Transportation.

Figure 1 shows a map of the village of Warmington, with the field site marked, and Figure 2 shows a plan of the site itself, with the position of the two 3m by 3m plots marked.

The substratum of the area is iron stone, an iron rich limestone, known locally as Hornton Stone. The soil is thus a rust coloured, neutral loam. In April 1991, a preliminary survey was done on the vegetation of the two study plots. The results of this survey are given in Table 3. The relative abundance of each plant species is measured as the number of 10cm by 10cm squares out of 125 such squares, in which the plant was

found. Over the years of the field work, the invasion of bramble into the two plots was controlled by pruning. The grasses in plot 2 increased significantly from 1991 to 1996.

Table 3: Relative abundance of plants in the two 3m by 3m plots.

Plant	Plot 1	Plot 2
Centaurea nigra	84	90
Plantago lanceolata	58	33
Vicia sativa	66	63
Glechoma hederacea	4	83
Veronica persica	0	25
Arum maculatum	5	9
Viola riviniana	0	7
Primula veris	86	5
Tarxacum vulgaria	0	5
Grasses	109	117
Mosses	122	120

Other plants identified in the plots:

Leucanthemum vulgare

Rubus fruticosus

Hedera helix

Above: The control plot in mid July 1991.

Below: The exclusion plot in mid July 1991, showing tent of nylon netting used to exclude gall flies.

FIGURE 1: POSITION OF FIELD STUDY SITE

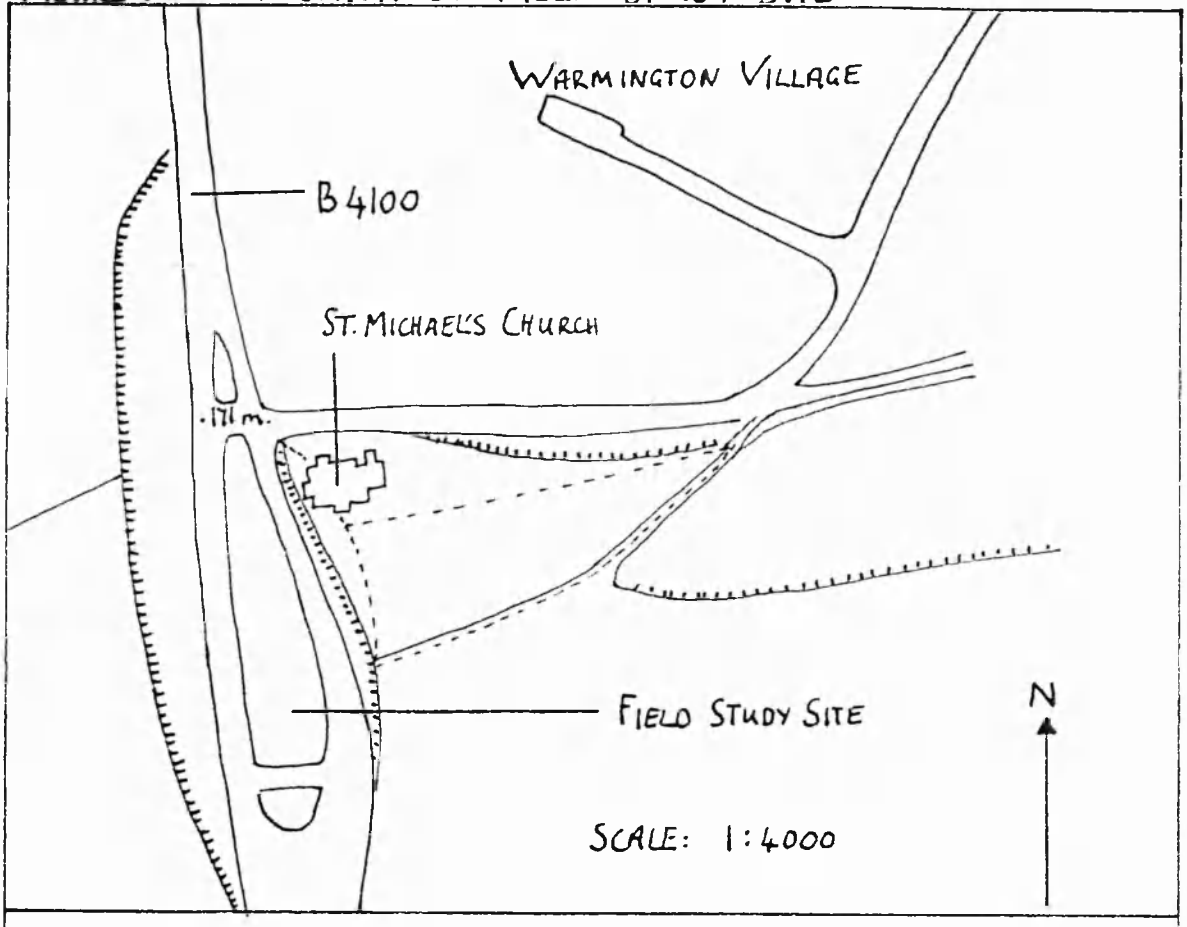
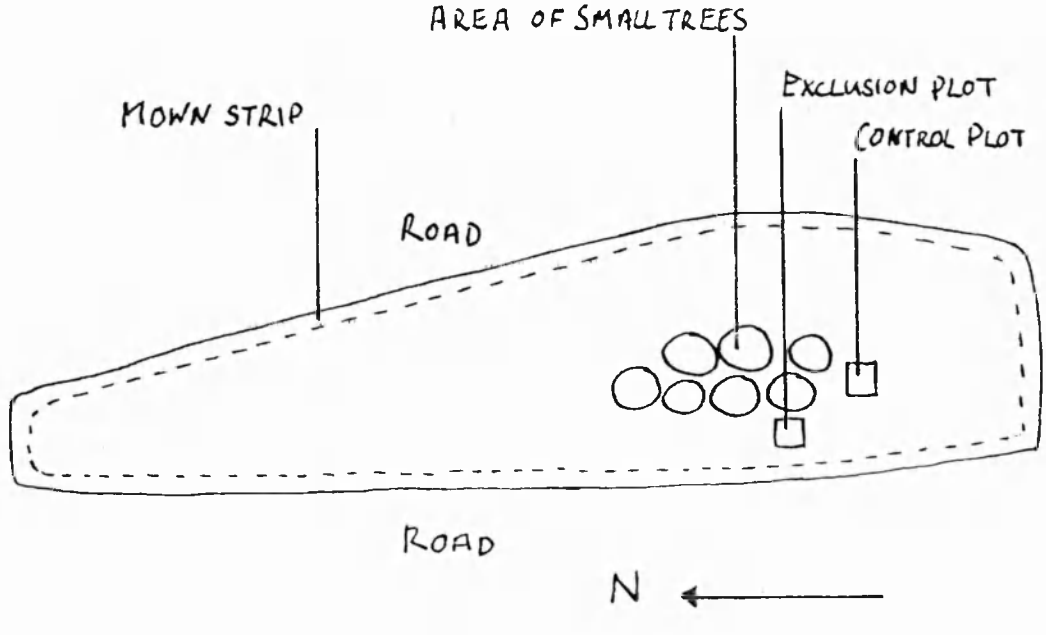


FIGURE 2 : FIELD STUDY SITE SHOWING EXPERIMENTAL PLOTS



Section 4:
General methods used in the thesis

2.4.1 Field work methods

Two plots measuring 3m by 3m were selected for similarity of plant density, aspect, and shading in the field site. For each year of field work, gall flies were excluded from one of these plots using a tent made of fine nylon curtain material erected over the plot for the period in which adult gall flies were alive. Access inside the tent to the plot was possible. The two plots were each marked out into nine permanent 1m by 1m quadrats, which were used to estimate flower density in the plots.

Field measurements of the size of flowerbuds on the day of budding were made using a pair of engineering external callipers to gauge the external diameter of the flowerbud, which was then read off using a steel rule.

Flowerheads were harvested in late August and September, when gall fly larvae were fully grown and before seed had fallen. In each year of field work, approximately 10% of flowerheads in plots were harvested.

2.4.2 Dissection of flowerbuds

In a number of experiments, the number of gall fly eggs oviposited in flowerbuds was counted. This could only be done by dissecting the flowerbud. First, the enveloping leaves were removed, then the bud was dissected through the centre from base to tip, and each half examined under a binocular microscope at x40 magnification. Eggs are easily

identified as creamy white, elongated, tapering to a point at both ends. They were found to be situated in the flowerbud just above the very small developing florets of the bud. Often, it was possible to distinguish between separate clutches laid in the same flowerbud, because clutches formed distinct groups of eggs. The most effective way to count eggs was to remove them from the flowerbud in a small drop of water, when they became even more visible.

2.4.3 Dissection of galls

One of the advantages of studying a gall insect, is that in this case, it lives all its life, apart from the adult stage, in the gall (Varley 1947; see also Redfern and Cameron 1978). On dissection of the gall, the contents of gall cells can be identified with relative ease.

Galls were first separated out from other flowerhead material, such as bracts, seeds, receptacle, and florets. Then using a dissecting scalpel, each gall cell was opened in turn.

Third instar gall fly larvae were identified by their segmented, creamy white bodies, facing downwards in the cell, with a darkened foot plugging the top of the cell. These larvae were quite stationary, except for slight movements of the head end. Second instar larvae were much smaller, and had often not turned round in the gall cell. They were also more active than third instar larvae. Pupae were identified in May dissections as brown skinned, oval shaped, with a darkened pigment at one end.

In autumn dissections, two parasitoids were easily identified. *Eurytoma curta*, as mentioned above, induces premature pupation in its host. Gall cells containing what appear to be a gall fly pupa have been parasitised by *Eurytoma curta*. If the skin of the pupa is broken, the fully grown larva of the parasitoid can be seen inside.

Torymus cyranimus can be identified as a single, very active, white larva with short hairs protuding from every segment, occupying a single gall cell.

Occasionally, gregarious parasitoids were found to occupy gall cells, and these were thought to be *Tetrastichus spp B*, although this was not confirmed.

Dissection of galls also revealed some cells in which there was no inhabitant, whether gall fly or parasitoid. Quite often, empty cells had evidence of frass in them. Empty cells were also associated with the discovery of a lepidoptera larva in the flowerhead.

2.4.4 Handling adult gall flies

Adult gall flies are easily identified in the field, with their characteristic striped wings, black abdomen and distinctive eyes. Observations of individual flies in the field were thus possible. As they also spend significant amounts of time stationary, they can be caught individually using a simple pooter, fitted with a glass specimen pot.

Marking of adult flies was attempted using a small spot of enamel paint on their abdomen, and this method was moderately successful (see Chapter 4.3).

In the indoor experiments on female adult behaviour, flies were kept in glass or clear plastic specimen pots with a strip of wet blotting paper at room temperature. Feeding of flies was not attempted (Varley 1947).

Extensive use was made of the fact that flies kept in pots oviposited as they were presented with suitable flowerbuds. A piece of thin white card, about 5cm square, with a hole in the centre just large enough to take the stem of a flowerbud, would be used to hold the bud. The card would then rest on top of a specimen pot containing tap water, into which the stem would reach. A specimen pot, containing a gall fly would then be inverted over the bud. In this way, chosen flies could be presented with selected buds.

2.4.5 Statistical methods

Data were organised using the Supercalc 5 spreadsheet programme, which was also used for the graphical analysis. This programme was used for the transformation and goodness of fit tests presented in Chapter 8.1. The remaining statistical analysis was carried out using the GLIM (Generalised Linear Modelling) programme, published by the Royal Statistical Society, with reference to Crawley (1993) and Sokal and Rohlf (1981).

The GLIM programme utilises the statistical concept of "deviance" (Crawley 1993) which is calculated using least squares or maximum likelihood methods, depending on the error structure of the test (Crawley 1993, Chapter 10.8: see Table 10.4). A number of link functions are available and data may also be transformed. In each analysis presented in this thesis the error structure, link function and any transformation will be indicated. GLIM also takes into account unequal sample sizes by weighting the calculation of deviance (Crawley 1993, Chapter 10.7).

Table 4: General form of the Analysis of Variance Table in this thesis.

	Sum of squares	Degrees of freedom	Mean Sum of squares	Fs
Among groups (Treatment)	Change in deviance	Change in d.f.	Change in deviance/d.f.	Fs
Within groups (Error)	Residual deviance	Residual d.f	Scale parameter	
Total	Total deviance			

In analysis of variance, the total deviance is calculated as the total sum of squares (of the difference between each data point and the mean) using the $Fit directive. When the effect of the treatment is added to the model, a change in deviance and a related change in degrees of

freedom results. The change in deviance is equivalent to the treatment sum of squares, and the residual deviance, ie. that which is not explained by the treatment, is equivalent to the error sum of squares. A scale parameter is also given, which is equivalent to the error variance. the F-ratio is then given by Fs = (change in deviance/change in degrees of freedom)/scale parameter. The procedure for linear regression is similar.

Table 5: General form of Analysis of Deviance table with Poisson and Binomial error structure.

	Scaled deviance	Degrees of freedom	X^2
Among groups (Treatment)	Change in scaled deviance	Change in degrees of freedom	Change in deviance
Within groups (Error)	Residual scaled deviance	Residual degrees of freedom	
Total	Total scaled deviance	Total degrees of freedom	

When a Poisson or Binomial error structure is specified (Crawley 1993, Chapters 14 and 15), GLIM calculates the deviance using the maximum likelihood method, and instead of the F-ratio, the test statistic is X^2. A Poisson error structure would be used for count data, and the binomial for proportional data. In both cases, the scale parameter is one. The

change in deviance resulting from fitting the treatment variable to the null model therefore gives X^2 (Table 5). In some cases over dispersion may occur with the Poisson or Binomial error structure, which may be corrected for either by calculating a scale parameter and fitting the model with the scale parameter (see Crawley 1993, chapter 14.9), or by transforming the data and using the identity link and normal errors, in which case the test statistic is the F-ratio.

Table 6: Form of table used to present results of significance tests in multiple regression.

Independent variable	Coefficient	Increase in deviance	Error variance	F-ratio
x(i)	b(i)	Increase in deviance after removing x(i) from the full model.	Scale parameter	Fs

The statistical methods used were mainly standard ones, using appropriate transformations of data and error structure, which are stated in the text. However, two sets of data required slightly more complex analysis. In Chapter 3.1, Nested Analysis of Variance was used to test for differences in flowerhead dry weights and seed production

between gall infested plants and non infested plants. In this analysis, differences between individual plants within the same treatment needed to be separated from the effects of the treatment on the individual flowerheads. Thus the experiment used a nested design. Unfortunately, the sample sizes were unequal, which made the significant testing slightly more complicated (Sokal and Rohlf 1981, Chapter 10, box 10.4).

In Chapter 12, multiple regression techniques were used in order to estimate the direct and stabilising selection gradients on some plant phenotypic characters. Each coefficient of multiple regression was tested for significance using the method described in Crawley (1993 Chapter 12.5). As each independent variable was removed in turn from the full model, the increase in deviance which resulted, was used to calculate the F-ratio, by dividing it by the error variance (scale parameter) (Table 6).

Part 2:
Individual Behaviour
and
Dynamic Modelling

Overview

The aims of Part 2 are:

1 To investigate individual insect behaviour in its own right.

2 To obtain reasonably realistic parameter estimates in order to develop a dynamic state variable model of individual female flies ovipositing in flowerbuds of the host plant.

3 To explore the features of the dynamic modelling equation.

The major decisions the gall fly has to make are:

1 where to search

2 which flowerbud hosts to accept for oviposition

3 the clutch size which is oviposited.

Chapters 4, 5 and 6 consider each of these questions in turn, in order to establish parameters for a dynamic modelling equation which is developed in Chapter 7. One of the predictions of the model is tested experimentally in Chapter 7.3. Population predictions are discussed in Chapter 8.

Chapter 3: Principles of Dynamic State Variable Modelling

In some population models the behaviour of organisms is an implicit feature (Hassell and May 1985). For example, including clumping of egg distribution in the case of an insect ovipositing on host plant patches (see Hassell and May 1985), implies foraging behaviour on the part of the insect, which gives rise to a greater or lesser degree of clumping. Any change in that behaviour may result in changes in the clumping distribution and therefore in the equilibrium density of the insect. However, such population models only incorporate summed population effects, and are sensitive only to changes in the behaviour of the average insect. In other words, these models do not incorporate individual differences, and whereas they may gain in generality, they lose a great deal of information by averaging out individual differences in, for example, foraging behaviour and oviposition site selection.

The class of models known as optimal behaviour models, rather than working from the population level, analyse decisions about behaviour in terms of their costs and benefits to the individual (Krebs and Kacelnik 1991). The basic assumption of these models is that behavioural decisions will maximise the benefits of the organism, offset against the costs of making a certain set of decisions. The model, therefore, incorporates a currency, for example, rate of food intake, risk of predation, or offspring survival. The constraints built into the model are to do with aspects of behaviour of the organism, for example its foraging or search efficiency, or handling time of prey. A decision variable defines the

choices which the organism may make in response to circumstances which it may encounter, for example, whether to eat or reject certain foods, or how long to forage in a patch.

Although simple optimal behaviour models do quantify the consequences of individual behaviour, they are seriously limited by treating only the present costs and benefits of the organism (Collier 1995, Krebs and Kacelnik 1991). For example, the model predicts that starlings foraging (Krebs and Kacelnik 1991) at different distances from the nest will adjust the amount of food they carry to the nest in each foraging period (the load size) in such a way as to maximise the energy gain to the whole family. However, what the model omits to do is to take into account the long term costs to the parents, and the effect on future offspring, of such foraging behaviour (Krebs and Kazelnik 1991).

Dynamic State Variable Models (Mangel and Clark, 1988; Mangel, 1987), overcome this limitation by taking as the common currency the sum of present and expected future lifetime fitness of the organism. The fundamental assumption of Dynamic Models is that the aim of the organism is to make decisions of behaviour which will maximise expected lifetime fitness (Mangel and Clark 1988). The expected lifetime fitness of an organism can be broken down into three parts: the fitness already accrued by decisions in the past; the fitness which may be gained in the present by making certain choices; the fitness to be gained in the future, based on an assessment of the present state of affairs. The third of these components, the future expected lifetime fitness, may influence the behaviour in the current time interval. In this way, the individual decisions made by the organism are not seen in isolation, but

affect future decisions within the life history strategy of the individual (Collier 1995) and will involve trade-offs between present and future gains in fitness (Heimpel and Rosenheim 1995).

A second feature of Dynamic State Variable Models, is that, in order to calculate the Expected Future Lifetime Fitness, certain state variables which describe the state of the organism, such as energy levels, egg load, risk of predation, age (Clark 1993, Collier 1995), need to be incorporated into the model. In addition it is possible to include variables, such as host density (Mangel and Clark 1988 Chapter 4), which describe the environment in which the organism lives. The crucial point here is that any decision an organism makes will change its state. So for example, the decision about how many eggs to oviposit in a particular host will determine by how much the egg load of the insect will be reduced as it enters the next time period (Mangel 1987); the decision about whether to forage in a less productive food patch, or to search for a better patch will affect the energy level of the animal (Mangel and Clark 1988 chapter 2). Decisions made will also change the environment, for example, an insect ovipositing in an unparasitised host will increase the number of parasitised hosts in a patch (Mangel and Clark 1988 chapter 4).

By incorporating state variables, Dynamic Models explicitly take into account variation between individuals in their physiological state, age, experience etc., rather than considering "the average individual". The variation seen in decision making concerning clutch size for example, is then explicitly related to the variation between individuals.

Other features of Dynamic Models are that they allow the state of individuals to change with time within the model, especially as these changes relate to decisions; they incorporate the variation resulting from the sequence of random events experienced by individuals (stochastic variation); and they can be tailored precisely to specific systems.

Other advantages of this type of modelling over other types of models are discussed by Clark (1993):

1 Parameters used in models can often be derived experimentally.
2 Predictions of the model, both quantitative and qualitative can be tested empirically.
3 Trade offs between different choices of the organism can be included in the model.
4 The effect of changes in the environment, for example, density of hosts, can be investigated.
5 The effect of changes in the state of the organism, for example, egg load, can be investigated.

The disadvantages of Dynamic Modelling are that it is hard to be sure that the global optimum is actually being modelled; that there is a lack of generality in the models; and that the computing time increases rapidly with the number of parameters which are included in the model.

In general, Dynamic State Variable Models begin by dividing the organism's available time into a number of time periods, the number and length of each time period depending on the system being modelled. The final period may be defined, for example, as the death of the organism,

or the end of a foraging day, whichever is appropriate for that particular organism. In that final period, the Expected Future Lifetime Fitness, by definition, is zero. The Maximum Expected Future Lifetime Fitness in each time period can then be calculated from that of the period after it, working back in time from the final period.

At the heart of each Dynamic State Variable Model is the computation of the Maximum Expected Lifetime Fitness of the organism in every time period of its life, and at all values of the state variables which are included in the model. The State Variables incorporated into The Dynamic Modelling Equation may refer on the one hand to the physiological state of the insect, such as the egg load, the age of the insect, or its energy level, and on the other hand to changing densities of hosts of different types which would affect the encounter rate with hosts (the environment). The encounter rate with hosts is also an important element in the equation, and will be determined by the density of suitable hosts, as well as by the search dynamics of the insect. The time intervals must allow for at most one encounter with a host, and also for no encounter with a host.

The computation is carried using the Dynamic Modelling Equation (Mangel and Clark 1988 chapter 2), which is of the general form:

$F(x_t,t,T)$ = the sum of:

[probable fitness gain in period t resulting from the optimal decision of the organism in each possible situation encountered in period t] + [the maximum expected future lifetime fitness in period t+1, at x_{t+1}]

where $F(x_t,t,T)$ is the maximum Expected Lifetime Fitness with the state variables of value x_t, in time period t, and with final period T, and x_{t+1} are the values of the state variables in time period t+1.

The result of this computation is an array of solutions of this equation for every time period and every value of the state variables. For each solution of the equation an optimal decision is included in the computation for each possible situation encountered by the organism. The optimal decision is by definition that which maximises Lifetime Fitness. For example, in the case of an insect searching for oviposition sites (Mangel, 1987; Mangel and Clark, 1988 chapter 4), the possible situations encountered in each time interval are:

1 not finding a host

2 finding a host of type i, where i is the number of different types of host available in the system.

The array of optimal behavioural decisions at different values of the state variables can be used to predict the behaviour of individuals (Mangel and Clark, 1988 Chapter 4). For example the State Variable Dynamic Models of Mangel and Clark were developed before convincing evidence of the influence of such variables as egg load was available. Indeed, one of the first predictions of these models was that egg load was an important factor in oviposition behaviour.

The array can also be used in conjunction with a Monte Carlo simulation exercise to quantify population level phenomena at different densities of organisms (see Chapter 8). Dynamic State Variable Models therefore work from the detailed modelling of behaviour of the individual organism in order to predict life history strategies, and population scale phenomena.

Examples of organisms that have been modelled in this way are the oviposition choices of the parasitic wasp *Nasonia vitripensis* (Mangel 1987), the density dependence of parasitism of tephritid fruit flies (Roitberg, Mangel and Tourigny 1990), and host feeding in *Aphytis melinus* (Collier et al 1994, Collier 1995). However, dynamic modelling is not confined to insects. Mangel and Clark (1988) include mammal predator-prey systems, and Clark (1993) gives examples of modelling birds, mammals and fish, as well as insects.

The task in hand is to apply the most appropriate dynamic model to the interaction between *Urophora jaceana* and *Centaurea nigra*. Female gall flies, searching for suitable flowerheads in which to oviposit, appear to reject some flowerheads and accept others, and also to lay more eggs in some flowerheads than in others. The ovipositional behaviour of female gall flies may be considered as a sequence of choices made by the insect in the course of its search for sites to lay eggs. The choices to be made by a female gall fly, for example, are whether to oviposit or not in a flowerhead which has been encountered, and how many eggs to lay in the flowerhead if oviposition is to occur. Present gain in fitness has to be set off against future possible gains, considering the likelihood of encountering a better site in the future, and also considering the number of eggs left to lay.

In subsequent chapters of Part 2 the search and oviposition behaviour of female gall flies is considered in order to establish realistic constraint parameters, such as the length of time intervals, and encounter rates with hosts (Chapter 4). Other parameters, such as life expectancy of adult females and initial egg load of adult females are estimated from the literature. The relation of components of fitness to clutch size, super-oviposition and host quality, is investigated, in order to establish an appropriate dynamic modelling equation (Chapter 6). A dynamic state variable model is then developed and the behaviour of the model at different host densities is investigated, and some of its predictions are discussed and tested (Chapter 7).

Finally, population level predictions of the model are explored using the Monte Carlo method (Chapter 8).

Chapter 4: Search and Foraging Behaviour of female *Urophora jaceana*

Overview

The aims of this chapter are:

1 To discuss the factors which influence the search and foraging behaviour of insects

2 To describe the main behaviour patterns of gall flies

3 To estimate the proportion of time the fly spends on each activity

4 To estimate the encounter rate of the fly with flowerbud hosts.

The methodology used in pursuit of aims 2, 3 and 4 was to make detailed observations of individual fly behaviour in the field, by following and noting the behaviour of individual flies.

4.1 Factors which influence search and foraging behaviour

The structure of the environment is critical in the search dynamics of insects. An insect searching for oviposition sites will encounter variable host availability and quality, in the patches in which it searches. Can information concerning host availability and quality be processed by insects, and are the choices it makes concerning movement from one patch to another, and utilisiation of hosts based on this information?

Experiments by, among others, Roitberg and Prokopy (1983, 1984), Mangel and Roitberg (1989), and Roitberg (1985), Rosenheim and Rosen (1991), on herbivores, and many experiments on parasitoids (see Godfray 1994) suggest that insects do have a limited capability of processing host information, and that this influences oviposition choices. After oviposition in *Crataegus* fruit, the female *Rhagoletis pomonella* (Tephritidae: Diptera) marks the fruit with an Oviposition Deterring Pheronome (ODP), which persists for at least three weeks (Averill and Prokopy, 1987). Three variables have been used to test the search behaviour of *Rhagoletis*.

Firstly, in an experiment on host deprivation (Fitt 1986) with three species of Tephritidae, it was found that the highly monophagous species did not attempt to oviposit in fruit outside their normal host range in the absence of their normal host, whereas the more polyphagous species did in fact choose hosts which were normally unacceptable. Roitberg and Prokopy (1983) have shown that the probability of *Rhagoletis pomonella* accepting hosts marked with ODP increases with the length of deprivation. It was found that after between 5 and 10 minutes of host deprivation, there is a significant increase in the tendency of the fly to accept marked hosts. If length of host deprivation gives an indication of host encounter rate, then at low encounter rates, one would expect oviposition behaviour to be modified accordingly. An important point about this experiment is that unlike the Fitt (1986) experiment, the deprivation times were so short that the increased acceptance of marked hosts cannot have been due to a build up of eggs during the time of deprivation, but must be a response to the flies' experience of hosts.

Secondly, experiments in which different sequences of ODP-marked and unmarked hosts were presented to *Rhagoletis pomonella* were used to investigate search persistence in patches (Roitberg and Prokopý, 1984). It was suggested by Roitberg and Prokopy (1984) that the Giving Up Time, that is the time before the insect gives up its search in a patch, may be related to the sequence in which it encountered marked and unmarked hosts. At the outset of searching in a particular patch, females would be expected to search for a set period, before giving up. This period is then adjusted up in response to encountering and ovipositing in unmarked hosts and adjusted down in response to encountering marked hosts. The results of this experiment are not conclusive. However, as only one larva can develop in a fruit, even if insects will super-oviposit, that is, lay eggs in a host in which there is at least one egg already, persisting in a patch with a higher proportion of marked hosts will result in lower reproductive success, than moving to a new patch with a lower proportion of marked hosts.

The third variable investigated is the Time Since Last Oviposition (TSLO) (Mangel and Roitberg, 1989). The propensity of an insect to oviposit in an already parasitised host was tested in relation to the fraction of marked hosts in the last five encounters and to the TSLO. The higher the fraction of marked hosts in the last five encounters and the greater the TSLO the more likely oviposition would occur in a marked host. The results of the experiment are very variable, and do not produce a simple correlation. Only by developing a model which takes into account the physiological state of the insect (egg complement and age) can the results be explained. It is possible that female *Rhagoletis pomonella* do respond to different physiological states and experience when selecting sites for oviposition. If the density of insects is relatively high, then it

would be expected that an individual would encounter a higher proportion of marked hosts in comparison to low densities of insects and, depending on its physiological state, may tend to super-oviposit.

These experiments do suggest that insects searching for suitable hosts modify their search and oviposition strategy in response to host availability and quality. Super-oviposition may be a response to low encounter rates with clean hosts, and also to the proportion of marked hosts in the patch. The search persistence in a patch may be modified by encounter rates with marked and unmarked hosts, and by oviposition in hosts. It was suggested by Mangel and Roitberg (1989) that an important factor influencing search dynamics of insects is certain state variables of the insect, which refer to the physiological state of the insect, such as its age, egg-load and recent experience.

Minkenberg, Tatar and Rosenheim (1992), reviewing how egg load, ie the number of mature eggs which are available for oviposition, influences insect foraging and oviposition behaviour, suggest that female insects with higher egg loads will search for hosts with greater effort, oviposit in less preferred hosts, spend less time in handling hosts, and oviposit larger clutches. Egg-load itself is a function of egg-maturation rate, egg capacity, and energy levels of the insect. Other factors, such as age, experience, and the risk of mortality between ovipositions confound the effects of egg-load. Separating the effects of egg load from the effects of these other factors is a major difficulty in investigating egg-load.

Rosenheim and Rosen (1991) have investigated the different influences of egg load and experience on oviposition decisions of *Aphytis lingnanensis* (Compere) (Homoptera: Diaspididae), an ectoparasitoid of scale insects.

Egg load was indirectly manipulated by insect size, and by cooling, thus overcoming the problem of confounding egg load and experience. Insects with smaller egg loads tended to take more time in locating a host, and in ovipositing than insects with larger egg loads. Moreover, both smaller egg load and prior experience of the insect in encountering hosts led to smaller clutches being oviposited. The implications of these findings are far-reaching in the consideration of search dynamics of ovipositing female insects. It may be the case that search efficiency, encounter rates with hosts, and oviposition decisions are influenced by the physiological state of the insect, and are themselves variables.

The use of oviposition deterring pheromones by ovipositing insects is one example of the use of olfactory stimuli in host search. Roitberg and Mangel (1988) discuss the benefits accruing to a female of marking hosts in which she has oviposited with an Oviposition Deterring Pheromone. It is assumed, very reasonably, that a single larva in a host has a better survival probability than hosts in which super-oviposition has occurred. In the case where time is limited, marking such hosts uses up valuable time. However marked hosts are more likely to be rejected than unmarked hosts. If a second oviposition can be avoided, whether by the same fly or by a different one, and if time is not limited, and there is a probability that more clean hosts will be encountered than the female has eggs, then there is an advantage in marking hosts. In the case where eggs are limited, ODPs will enable a female, whether the same or a different one, to evaluate those hosts in which oviposition has already occurred. The identification of marked hosts is advantageous to the ODP producer, if survival of single larvae is greater than when other larvae are present in the same host.

The parameters determining the deployment of visual stimuli have been investigated by Roitberg (1985) in attempting to quantify the reactive envelope of *Rhagoletis pomonella*. As a fly moves through a patch, it will respond to visual stimuli within a certain space surrounding the insect, which is its reactive envelope. Roitberg (1985) suggests that the size of an insect's reactive envelope is a factor which contributes to its search efficiency, and also the nature of its search paths.

To summarise, the dynamics of an insect searching for hosts are a complex interaction of many factors which influence the search and oviposition strategy of the insect. The structure of the habitat, and what constitutes a patch, the density of suitable hosts of different quality, the physiological state of the insect, especially its egg load, its reactive envelope to visual stimuli, its current experience of encounters with hosts and its search path pattern, all contribute to the system. Isolating one factor from the others, in order to break down the system into components, can help to evaluate the parameters that should be attributed to that factor in a particular case.

4.2 Search and Oviposition Behaviour of female *Urophora jaceana*

Varley (1947) records that oviposition by female *Urophora jaceana* is only rarely observed in the field. In the course of one morning he observed five females oviposit a total of eight times. Gall flies are often seen to be resting on *Centaurea nigra* leaves for as much as two hours at a time. When mating occurs, it can last over 45 minutes. A single oviposition may last about five minutes. In the time remaining, the gall fly exhibits a small range of behaviour, most of which may be related to searching for available hosts in which to oviposit.

Varley (1947) notes that weather conditions affect the behaviour of *Urophora jaceana* in the field. A peak of activity is reached around 20°C. Oviposition was observed at temperatures above 16°C, but not below that level. Both temperature and humidity appear to affect the total fecundity of females, with the highest fecundity found when flies were kept between 22°C and 32°C and above 60% saturation. Above and below those temperatures the fecundity falls off.

Here, detailed observations of individual females are employed in order to gain an insight into the behavioural patterns of *Urophora jaceana* in the field, in order to make the dynamic model developed later in this chapter more realistic.

4.2.1 Method

Observations were made on seven separate days between 20th June and 12th July 1994, in and around the field study site in Warmington. In this period, the weather was dry and warm, with a light to moderate breeze. Altogether, observations of 11 individual female flies were made, and also of any males that they encountered. The length of time spent observing individuals ranged from 10 minutes to 114 minutes, and the total observation time over the seven days was 12 hours 35 minutes. Flies were observed until they disappeared from sight or to the end of an observation session. Observation periods included early morning, mid morning and early afternoon.

As a fly was observed, the different elements of behaviour, and the time of occurrence and duration of that behaviour were recorded. The types of behaviour observed included:

1 Behaviour while stationary, such as turning, looking from side to side, preening, extending ovipositor, and also the position of the wings.

2 The manner of movement, whether walking or flying. In the case of flying, the distance flown, and in the case of walking the direction of movement were recorded.

3 Encounters with flowerheads of *Centaurea nigra*, noting the developmental stage of each flowerhead encountered.

4 Oviposition and pre- and post-oviposition behaviour.

5 The nature of encounters with other individuals, particularly interference from other females, and mating with males. Males were also observed encountering other males.

4.2.2 Results

1 **Behaviour while stationary.**

When inactive, male and female *Urophora jaceana* usually placed their wings parallel with the body, and remained stationary, but for the occasional movement on a leaf or flowerhead of *Centaurea nigra* for significant periods of the day. Four females were observed in this position for a mean period of 61 minutes at different times of the day.

At other times, stationary female *Urophora jaceana* exhibited a range of behaviour. Wings were normally held at an angle of between 45^0 and 90^0 to the axis of and in the same plane as the body. Preening of legs, wings, ovipositor and head was undertaken by both front and back pairs of legs. This was observed a number of times after the insect had walked over cuckoo spit, or honey dew, and after mating and ovipositing. Female *Urophera jaceana* were also observed touching the surface of a stem or leaf with the proboscis, as if feeding or drinking.

The insects were observed to turn on the spot, whether on a leaf or a flowerhead of *Centaurea nigra.* They also raised their heads by stretching the front pair of legs, and squatting back on the back pair, and moved the head and thorax in an arc of about of about 45^0. Usually this was observed to occur when the insect was at the tip of a leaf, and was often the prelude to movement, whether walking or flying.

2 The manner of movement.

Male *Urophora jaceana* were observed not to move very much. They tended to position themselves in a prominent position on a leaf or flowerhead and stay there, turning, preening and looking. Female *Urophera jaceana* on the other hand were much more mobile. When searching, flight occured at a frequency of about one flight every 2 or 3 minutes, and varies in distance between 3cm and 40cm. Longer flights of up to 150cm were observed occasionally. The mean length of flight was 13.85 cm (standard error 1.26) in 94 observed flights. Take off was usually from the tip of a leaf, or occasionally from a stem or a flowerhead. Landing was usually on a stem, mostly of *Centaurea nigra,* but also occasionally of other species of plants. The position of take off

was usually higher than the position of landing. It was by flying that female *Urophera jaceana* were often observed to move from one plant to another while searching.

After landing on a stem of *Centaurea nigra* the female *Urophora jaceana* immediately walked up the stem to the highest bud, walked over the bud, and then walked down to the first branch of the stem, and up to the second bud. Flies were observed on a number of occasions to proceed in this manner, even down to the fourth bud on the stem. Sometimes the fly would retrace its steps so that a particular bud would be visited two or more times.

Depending on the availability of suitable routes, flies also walked from one stem to another, via adjacent leaves, or by stems, leaves of grass, dead stalks, which criss-cross the upright stems of *Centaurea nigra*.

On occasions it was noticed that cuckoo spit acted as a barrier on stems of *Centaurea nigra*, because flies were reluctant to walk over it.

3 Encounters with flowerheads.

Female *Urophora jaceana* on reaching a flowerbud walked over the bud, possibly returning a number of times to the same one. The enveloping leaves appeared to be an important feature which flies investigated. If they more than half enveloped the bud, then they presented a physical barrier and the fly had difficulty in gaining access to the bud itself. Larger buds representing a later stage of development, when the bracts are well protruding were not extensively investigated when encountered by flies. Those buds which were investigated by the flies were those

where the enveloping leaves allowed access to the bud, but where the bracts were not protruding. Flies were observed on occasions to "jack-knife" on these buds and to probe in different places, without extending their ovipositors.

4 Oviposition behaviour.

Having assessed the status of a flowerhead, by walking over it, and by probing in different places with the ovipositor, female *Urophora jaceana* were observed to oviposit. The ovipositor was extended into the flowerhead and remained in that position for between one and six minutes. A slight pumping action could be observed in the abdomen. On withdrawal of the ovipositor, which remained partially extended, the insect sometimes dragged the ovipositor over the flowerhead. Although difficult to see in the field, it was just possible to observe a clear fluid being secreted from the end of the ovipositor. On two occasions, the ovipositor was retracted, and did not mark the flowerbud. The post-oviposition period was taken up by preening, before the fly moves from the flowerbud to a leaf or stem. On one occasion, mating was observed immediately prior to ovipositing. Eight other instances of oviposition were observed, but with no mating occuring immediately beforehand.

On one occasion, super-oviposition was observed directly. A female *Urophora jaceana* having oviposited once in a flowerhead without having marked it, returned less than a minute later and oviposited again in the same flowerhead. On dissection, two batches of eggs were discovered, one of five and one of two eggs.

A number of flowerheads in which flies had been observed to oviposit were dissected, and batches of two, three, four and five eggs were discovered.

5 Encounters with other females.

Encounters with other females happened rarely in the field because of the low densities of flies. It was observed once directly, when the fly being observed arrived on a flowerhead beneath which another fly was already positioned, and was getting ready to oviposit. The fly which was there first moved towards the newcomer and the latter promptly flew off into a completely new patch. This interference cost this fly a total of seven minutes. Moving to a new patch, and searching for a new flowerhead in which to oviposit, accounted for this time.

6 Encounters with males.

Four direct encounters with males were observed, of which three led to mating. On detecting an approaching female, the male moved towards her, and jumped upon her back with no observable courtship. The male's back legs held the female's ovipositor, which angles itself so as to penetrate the male's genital region at the end of the abdomen. Mating was observed five times, with mean duration of 22 minutes, although on one occasion mating was in process when observation began, and on another, was still in process when observation ended. A mating female may walk or fly. On one occasion, a pair flew a short distance to avoid another male.

Males were observed not to move a great deal, but when they encountered another male, a confrontation ensued, which ended with one of the males flying off. This may suggest that males are territorial, guarding a suitable oviposition site so that a female will have to mate in order to gain access to the site.

One female had been mating for 42 minutes, after which she walked, with the male still on her back, possibly guarding to ensure paternity, to the nearest flowerhead, where she oviposited. After oviposition had been completed, the pair resumed mating for a further 14 minutes.

On one occasion the female was observed to avoid a male. This particular female was actively searching for a flowerhead in which to oviposit, and rather than mate, on encountering the male flew off to another plant, where she resumed her search, and indeed did oviposit shortly after.

7 Mean duration of different activities of female *Urophera jaceana*

The mean duration, and standard error, spent by female *Urophora jaceana* on each activity, were calculated, from data on 14 individual flies, observed for a period greater than 30 minutes each (Table 7).

A search period is defined as beginning when an insect flies from one stem of the host plant to another, and ending when it either leaves the stem, or when it oviposits, or when it mates.

The oviposition period includes both pre- and post-ovipositional behaviour from the time the fly arrives at a flowerhead until it leaves. Mating is the duration of copulation. A stationary period is when a fly,

which has been observed to search, stays in one place. An inactive period is when a fly remains in the same position for the whole of the observational period.

Table 7: The mean length of period female *Urophora jaceana* spend on different activities. Means with the standard errors, are calculated from data on 11 individual flies. The number of observations, the total time spent, and the percentage of the total observational time spent on each activity is also shown. For definitions of length of period, see text.

Behaviour:	Mean duration (Minutes)	(S.E.)	Number of observ-ations	Total time on each activity	% time on each activity (excl. inactive)
Inactive:	63.0	(11.0)	4	252	
Stationary:	21.8	(2.8)	4	87	17.8
Mating:	33.7	(12.1)	3	101	20.7
Searching:	3.6	(0.4)	66	238	48.7
Ovipositing:	7.6	(0.7)	9	56	11.4
Interference:	7.0		1	7	1.4
Total excluding resting				489	100

Table 8: Activity of a female gall fly, observed on 2nd July 1994

Activity	Time began	Time ended	Duration (minutes)
Ovipositing	9.11	9.16	5
Searching	9.16	9.26	10
Ovipositing	9.26	9.30	4
Searching	9.30	10.11	41
Ovipositing	10.11	10.17	6
Searching	10.17	10.36	19
Ovipositing	10.36	10.46	10

Table 9: Number of flowerbuds visited, total time taken and time between visits for six female *Urophora jaceana*.

Fly	Number of visits	Total time (minutes)	Time between the beginning of one visit to beginning of next
1	12	74	6.16
2	12	30	2.50
3	7	38	5.43
4	28	107	3.82
5	12	45	3.75
6	33	117	3.54
Total	104	411	Mean 4.20 s.e 0.50

The sum of the periods for each activity is also shown. The percentage of observational time which ten of the flies together (excluding the four which spent the whole observation time inactive) spent on the different activities is calculated as the sum multiplied by 100 divided by the total observational time.

Female flies spent 34.0% of the time resting. Searching and ovipositing together account for 60.1% of the activity of flies. The frequency of oviposition was no more than one oviposition in 15 minutes. Resting did not necessarily follow oviposition. An example of an actual sequence of searching and ovipositing is given in Table 8. The number of flowerbuds, at all stages of development, visited by female flies, was also noted. The mean of the time between between visits for the six flies is 4.2 minutes with a standard error of 0.5 (Table 9).

4.2.3 Discussion

The analysis of the mean duration of the different activities of female *Urophora jaceana* reveals that the flies observed were active for a little under two thirds of the total time spent observing. There may be different explanations for the periods of resting which were observed. Varley (1947) notes that females only begin to oviposit about three days after they have emerged. During this time, flies may rest a significant amount of time.

Varley (1947) also noted that temperature influences the activity of gall flies. As already noted, at temperatures lower than 16^0 C females tend not to oviposit. This is especially important overnight, and in the early morning and late evening, when temperatures are lower, and which are

therefore probably periods of inactivity. However, observations were not made at these times. Also, according to Varley (1947), at temperatures above 24^0 C, flies tend to reduce their activity again. The temperature at which peak activity occurs appears to be around 20^{0}C. Varley found that flies in the field were thus most active during the morning, on those days when temperatures rose above 24^0 C. It is thus possible that inactivity is associated with both high and low temperatures. Some of the periods of inactivity observed in the field may have been determined by the temperature.

Oviposition deterring pheromones (ODP) may play a role in oviposition decisions. Observations in the field suggest that in some cases hosts are marked with an ODP following oviposition, but that in other cases no such marking takes place. If ODP-marking is associated with the avoidance of super-oviposition, it may be that it is more likely to take place when eggs are in short supply and clean flowerbuds are in plentiful supply. Super-oviposition does, however, occur and has been observed in the field, when a female oviposited once in a flowerhead, and a few minutes later went back to the same flowerhead to oviposit a second time. In the oviposition choice experiment described in Chapter 5, females were found to oviposit up to five times in the same flowerhead. Furthermore, the typical clutch size oviposited has been observed to be between two and six eggs, whereas some flowerheads have been found in the course of sampling with over twelve gall cells in them (see Chapter 6, section 2). Such large numbers of gall cells are likely to be the result of super-oviposition.

The search behaviour of *Urophora jaceana* as observed in the field suggests that visual stimuli (Roitberg 1985) are also important. A searching female stands on the tip of a leaf of the host plant, with its wings at right angles to the longitudinal axis of its body and in the same plane as the body. The insect then raises itself high on its front legs and moves its head in an arc of about 45°. This is often the prelude to its taking flight and landing on a new stem of *Centaurea nigra*. The length of individual flights of *Urophora jaceana* is, in 90% of flights, between 3cm and 30cm, which suggests that its field of vision may be limited to a maximum radius of about 30cm, or alternatively that it is not a very strong flier.

Female *Urophora jaceana* spend about 60% of their active time searching for suitable hosts and ovipositing in these hosts. Searching proceeds by moving, usually by flying, and occasionally by walking, from one stem to another, and searching the flowerbuds of each stem for suitable oviposition sights.

Chapter 5: Which flowerbud hosts are accepted for oviposition?

The aim of this chapter is to ascertain which flowerbud hosts are preferred by the female gall fly for oviposition. Flowerbuds at different stages of development were presented to individual flies, and the observed frequency of oviposition choices were compared to the expected frequency of choices with no preference.

5.1 Oviposition strategy

Species of Tephritidae (Diptera) ovipositing in flowerheads, generally of the Compositae, deploy a number of strategies in order to exploit food resources available in the flowerheads. Straw (1989b) suggests three strategies of attack, which are typical of different tribes of the Tephritidae.

Strategy 1 is early attack and general feeding. Oviposition occurs at an early stage of flowerhead development, before flowering. The larvae begin to feed in the immature achenes, and then move on to other flowerhead tissue. Highly developed gall structures are not produced. This strategy is deployed within the whole range of species of Compositae, with flowerhead sizes ranging from small to large, and is typical of species of the group Tephritini.

Strategy 2 is late attack. Oviposition occurs at a late stage of flowerhead development, post flowering, when the achenes are filling out. Achenes must be large enough in this instance to provide sufficient

nutrients for larval development, associated with species of Compositae with larger flowerheads. Species of the genera *Terellia* and *Chaetorellia* feed more generally on the mature achenes, moving from one to another when the larva has exhausted the resources of the first.

Strategy 3 is early attack, associated with the production of well differentiated galls, by larvae of the subfamily Myopitinae. Oviposition occurs at an early stage of flowerhead development, as in strategy 1, but unlike the Tephritini, which exploit the nutrients available in normal flowerhead development, in this case the larvae burrow down into floret tubes and induce gall production in the developing achenes and receptacle. Gall production entails a diversion of plant resources away from other plant structures into the gall, thus increasing the availability of plant nutrients to the larvae.

Straw (1989b) suggests that each of these strategies exploits flowerhead structures at times when their growth rate is at a maximum. Thus the larvae of the early strategist *Tephritis bardannae* (Schrank) feed in flowerheads of *Arctium minus* (Compositae) at a time when the receptacle and the floret tubes are growing most rapidly. The larvae position themselves in the developing floret tubes where nutrient flow is presumably greatest. This compares with the larvae of the late strategist *Cerajocera tussilaginis* (Fab.), which position themselves and feed within single achenes at a time when achene growth is at a maximum.

Straw (1989b) concludes that the timing of oviposition is critical for all three strategies, in order for Tephritidae larvae to exploit the specific plant structures as they become available. Ovipositing females must utilise a range of mechanisms for evaluating hosts at a suitable stage of

development. Comparing bodily dimensions with internal or external dimensions of host flowerheads is one possible mechanism. Alternatively, the body size of the female may physically limit its capability of ovipositing in flowerheads over a certain size (Straw 1989c).

In this scheme of things, *Urophora jaceana* exploiting flowerheads of *Centaurea nigra* deploys strategy 3. The larvae produce highly differentiated galls, and oviposition occurs early in flowerhead development. Varley (1947) notes that oviposition occurs in flowerheads of between 3mm and 5mm diameter: very few ovipositions occur in flowerheads greater than 5mm, and in flowerheads of less than 3mm, where the buds have not emerged from their enveloping leaves. Furthermore, the stage of development of larvae of *Urophera jaceana* in the field was closely related to the stage of development of the flowerhead (Varley, 1947).

An experiment was devised in order to determine the stage of development of flowerheads of *Centaurea nigra* in which female *Urophora jaceana* are more likely to oviposit. Since it is established that oviposition occurs at an early stage of development (Varley 1947, Dempster et al 1995a), the aim of the experiment was to define that early stage more precisely.

5.2 Method

Female gall flies were collected on the day of emergence from galls over-wintered in 20mm diameter glass specimen pots. Each female was kept for three days (including day of emergence) with one male in a separate pot. This was to attempt to ensure that all females in the

experiment were ready for oviposition and had the same experience. By using virgin females it was hoped that the egg load would also be similar. On the fourth day a set of five selected flowerheads, arranged in the following manner, was presented to the female.

Five plastic tubes, 80mm long and 5mm in diameter were sealed at one end, and fixed vertically in a standard configuration on a flat whit surface. Four tubes were placed in a square of sides 35mm, and the fifth was placed in the centre of the square. The tubes were filled with tap-water. Four similar arrangements were set up, to enable concurrent trials to take place, depending upon availability of flowerheads.

For each trial of the experiment, five fresh flowerheads of *Centaurea nigra* were selected from plants grown in pots and kept in the open, free of *Urophora jaceana*. Table 10 shows the size range and stage of development of the selected flowerheads.

Table 10: Flowerhead size and stage of development of flowerheads selected for oviposition choice experiment.

Category	Flowerhead diameter	Stage of development: % of bud enveloped by leaves	
1	< 4.0mm	100%	no bracts protuding
2	4.1mm to 4.5mm	75%	no bracts protuding
3	4.6mm to 5.5mm	50%	no bracts protuding
4	5.6mm to 6.5mm	25%	bracts protuding
5	> 6.5mm	0%	bracts protuding

These categories of flowerhead were selected in order to relate to stages of early flowerhead development. Category 2 relates to the time of budding, and category 4 to the time when bracts begin to protude. Category 1 is the pre-budding stage, and category 5 is post-bract protusion. Category 3 relates to midway between budding and bract protusion.

The flowerheads were picked with sufficient length of stalk to be placed in the tubes, so that the five flowerheads were all at the same level. Flowerhead categories 1,2,3, and 4 were placed at the corners of the square and flowerhead category 5 in the centre. This arrangement was kept constant for all trials of the experiment. This arrangement, unfortunately, gives rise to the potential problem of edge effects. The seriousness of this problem is discussed below (see discussion). Randomising the position of flowers would also have reduced any bias in the experimental design.

For each trial the five flowerheads with a single female gall fly were contained in an upturned, clear plastic jar, 200mm high and 100mm in diameter. The set-up was left in diffused natural light, indoors, at a range of temperatures between 18°C and 22°C for a period of eight hours.

After eight hours the gall fly was removed, and the five flowerheads were dissected under a binocular microscope (eyepiece x10, objective x2). Eggs were found to be grouped in distinct clutches within each flowerhead. The number of eggs in each clutch, and the number of clutches within flowerheads were therefore recorded, as well as the total number of eggs.

32 trials of the experiment were carried out, between 17th June and 4th July 1993.

5.3 Results and analysis

Table 11: The number of clutches found in each category of flowerbud.

Bud Category (mm) Trial:	< 4	4.1 - 4.5	4.6 - 5.0	5.6 - 6.5	>6.5
1	0	0	4	2	0
2	0	1	3	1	0
3	2	2	0	2	0
4	0	2	4	1	0
5	0	1	2	0	2
6	1	2	4	1	0
7	0	0	4	1	0
8	0	0	2	1	4
9	2	1	5	3	4
10	3	2	5	1	0
11	0	0	3	0	0
12	4	0	3	1	0
13	2	1	2	1	0
14	0	0	0	1	2
15	0	0	3	0	0
16	1	4	5	0	0
Total:	15	16	49	15	12

Of 32 trials of the experiment, 16 female gall flies oviposited (Table 11). Eggs were generally laid in clutches of between 2 and 5 eggs. The frequency of clutches, rather than the frequency of eggs, in each flowerhead category, is tested for goodness of fit against the expected frequency of equal number of clutches in each category, because the eggs laid within a clutch are not independent events.

The GLIM programme was used, with the Poisson error structure and identity link, to fit the number of clutches per flowerbud against flowerbud category (Table 12). The amount of deviance explained by flowerbud category (X^2) is 36.68, which is significant at $p << 0.001$ with four degrees of freedom. The number of clutches laid in different categories of flowerhead is therefore significantly different. When category 3 flowerbuds are removed from the test, the amount of deviance explained (X^2) is 0.56, with 3 degrees of freedom. There is therefore no significant difference between the remaining categories (Table 12).

In view of the possibility of edge effects, the test was repeated excluding flowerbud category 5, the central bud, leaving the four categories in equivalent positions (Table 13). In this case, the amount of deviance (X^2) explained by flowerbud category is 30.76, with 3 degrees of freedom including category 3, which is significant at $p << 0.001$. When category 3 is removed, the value of X^2 is 0.043 with 2 degrees of freedom, which is not significant.

Table 12: Results of fitting number of clutches per flowerbud against flowerbud category: all five categories included.

	Deviance	df	X^2 (G)	Sig
Among categories	36.68	4	36.68	$p < 0.001$
Error	116.46	75		
Total deviance	153.14	79		
Omitting category 3:				
Among categories	0.56	3	0.56	ns
Error	86.88	60		
Total deviance	87.44	63		

Table 13: Results of fitting number of clutches per flowerbud against flowerbud category: category 5 excluded.

	Deviance	df	X^2 (G)	Sig
Among categories	30.76	3	30.76	$p < 0.001$
Error	81.76	60		
Total deviance	112.60	63		
Omitting category 3:				
Among categories	0.04	2	0.04	ns
Error	64.19	45		
Total deviance	64.23	47		

5.4 Discussion

The experiment confirms that female gall flies oviposit in flowerheads of *Centaurea nigra* at an early stage of development, and will oviposit in the whole range of categories of flowerheads employed in this experiment. However, since the frequency of clutches laid in category 3 flowerheads, that is in flowerheads between 4.6mm and 5.5mm in diameter, and with the flowerbud approximately 50% enveloped in leaves, is significantly higher than in the other categories, it is probable that gall flies select such flowerheads when they are available for oviposition, in preference to the other categories.

A serious flaw in the design of this experiment, as noted above, is in the possibility of edge effects in the arrangement of buds. It is possible that flies avoided the central flowerbud, and preferred the outer flowerbuds, not by virtue of the category of bud, but because of the position they occupied in the design.

Unfortunately, because of the shortage of flies in subsequent years, it was not possible to repeat this experiment with a design which avoided this problem. However, testing for difference in number of clutches in the four outer categories of flowerbuds, with the central category removed, indicates that one of the outer buds was preferred over the three other outer buds. The edge effects would have acted in the same way for each of the outer buds, because each of them occupy an equivalent position to the others. Despite the possibility of edge effects, the result obtained does appear to be sufficiently robust.

In conclusion:

1 It is confirmed that flowerbuds in an early stage of development are chosen for oviposition.

2 The preferred stage of development is when flowerbuds are more than 50% uncovered by enveloping leaves, but before the onset of bract protusion.

3 Oviposition will occur in flowerbuds in earlier and later stages of development than those which are preferred stage.

Chapter 6: Clutch Size and Larval Mortality

Section 1: Clutch size in insect life history strategy

In this section clutch size as a component of life history is discussed in relation to:

1 The Lack Solution clutch size, which assumes that an organism will maximise fitness per clutch.
2 Clutch size in insects.
3 Clutch size in relation to host quality.
4 Some of the methodological problems of studying clutch size in insects.

6.1.1 The Lack Solution clutch size

As a component of an organism's life history strategy, clutch size is a classic optimisation problem, and is likely to be a product of response to several selection pressures (Godfray 1987).

Beginning with Lack (1947), the optimal clutch size of birds was taken as that which maximises parental fitness per clutch. If the fitness return of the clutch is plotted as a function of the clutch size, then the optimal clutch size is that which gives the maximum fitness return. Expressed mathematically, if $w = f(c)$, where w is the fitness return per clutch, and c is the clutch size, then $dw/dc = 0$ when $c = c^*$, the

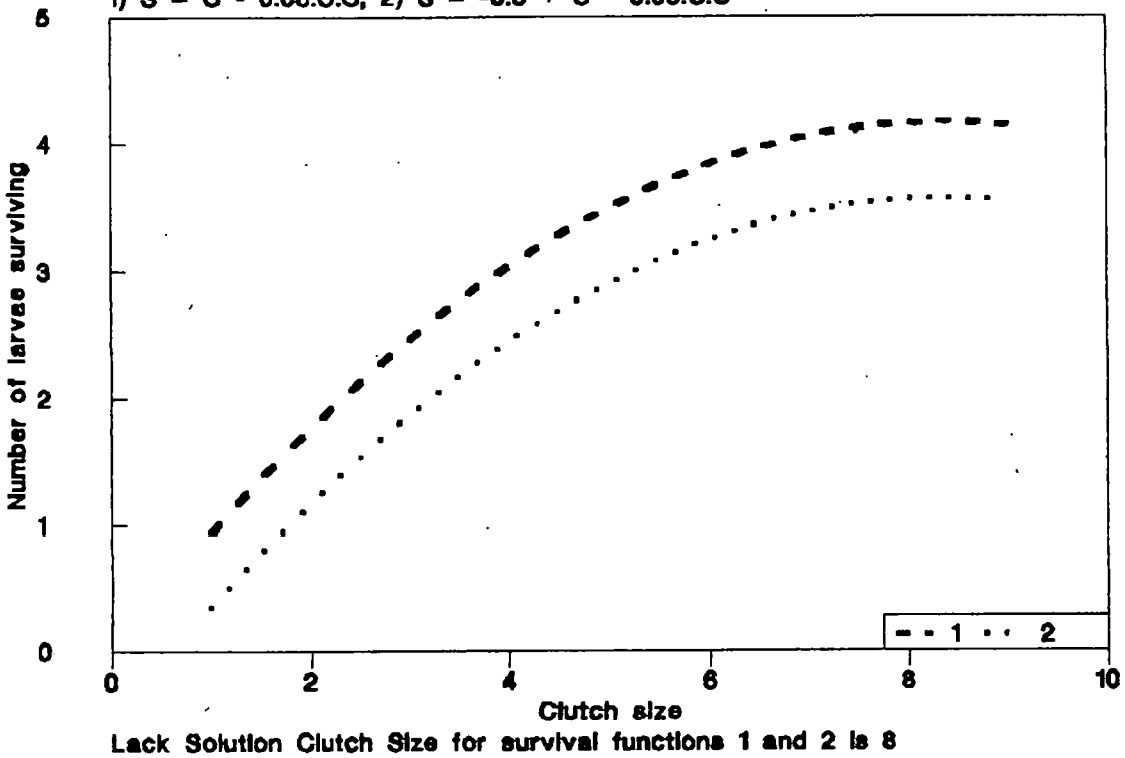
optimal clutch size. This relationship is shown graphically in Figure 3, where the optimal clutch size can be read off at the maximum of the fitness curve. This model has become known as the Lack Solution Clutch Size Model (Charnov and Skinner 1984; Godfray 1987).

An alternative way of expressing the relationship is to consider the fitness increment per egg in clutches of different size. Fitness return per clutch is then the fitness increment per egg multiplied by the number of eggs in the clutch. The fitness gain per egg may be independent of clutch size, decrease monotonically, that is it decreases as a function of clutch size, increase monotonically with clutch size, or increase, reach a maximum, and then decrease at larger clutch sizes, giving rise to a dome shaped relationship with clutch size. These patterns are illustrated in Figure 4.

6.1.2 Clutch size in insects

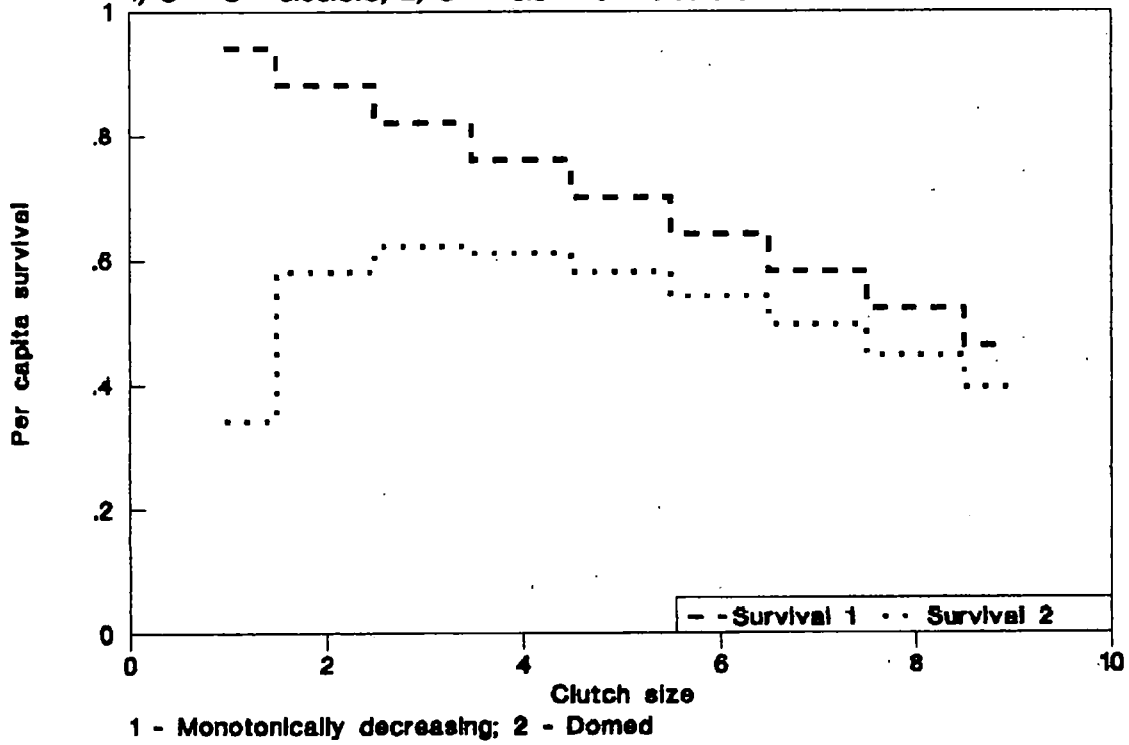
Godfray (1987) lists a number of studies on insects, which have related per capita fitness increments to clutch size. Some show the monotonically decreasing pattern, mainly in the Hymenoptera and Coleoptera families. Clutch sizes of one to the Lack Solution are possible with this pattern. Others show the domed pattern, such as *Pegomya nigritarsis* (Diptera: Anthomyiidae). Here, the smallest clutch, except with severe egg limitation, would be the clutch size at which per capita survival is at the top of the dome. Other species show a monotonically increasing pattern, notably three in the Lepidoptera, for example *Lymantria dispar*. In the monotonically increasing pattern, there is effectively no Lack Solution Clutch size.

Figure 3: Lack Solution Clutch Size I
Number surviving per clutch plotted against clutch size for two clutch size survival functions.
Survival functions:
1) S = C - 0.06.C.C; 2) S = -0.5 + C - 0.06.C.C



Lack Solution Clutch Size for survival functions 1 and 2 is 8

Figure 4: Lack Solution Clutch Size II
Per capita survival plotted against clutch size for two clutch size survival functions
Survival functions:
1) S = C - 0.06.C.C; 2) S = -0.5 + C - 0.06.C.C



1 - Monotonically decreasing; 2 - Domed

Godfray (1987) discusses the trade-offs between maximising fitness per clutch, assumed in the Lack Model, and future reproduction. He points out that empirical studies on parasitic wasps (Waage and Godfray 1985) show that observed clutch sizes are usually less than the Lack Solution, suggesting that maximising fitness per clutch is not a strategy which optimises the lifetime fitness of the parent. In the case of a monotonically decreasing fitness per capita, increasing clutch size brings diminishing returns. If for example, the number of eggs available to oviposit is limited, if time is unlimited, and if there are sufficient oviposition sites, then the strategy to maximise lifetime parental fitness would be to oviposit clutches smaller than the Lack Solution. The key concept here is the optimising of lifetime expected fitness of the parent, rather than maximising fitness per clutch.

In Mangel and Clark (1988), one example given is that of *Nasonia vitripennis* (Hymenoptera: Pteromalidae) studied by Charnov and Skinner (1984, 1985). This parasitic wasp has a monotonically decreasing pattern of per capita fitness in relation to clutch size. Here fitness per clutch is a cubic function of clutch size. It is the curvature of the function which is the key element in the Dynamic Modelling Equation which Mangel and Clark develop for this organism. Where the fitness function has curvature and a maximum, there is the possibility of a trade off between the present gain in parental fitness and the gain expected in the future. Expressed economically, it is a choice as to whether to invest now, or to save for the future.

6.1.3 Host quality and fitness

Variation in the fitness of a clutch may also be attributable to variation in host quality. In the case of *Nasonia vitripennis*, mentioned above, a different fitness function was obtained for four hosts of different volumes, (Charnov and Skinner, 1984, 1985). Mangel (1987) quotes Carey and Freeman, who found that the number of eggs laid by the medfly is fewer in hosts of 1.5cm diameter, averaging at two eggs per host, and more in hosts larger than 3 cm diameter, averaging between three and four eggs, although Mangel does not mention whether survivorship in small hosts is any less than in large ones. Tatar (1990) found inconclusive evidence that the swallowtail butterfly, *Battus philenor*, adjusted clutch size according to host quality. Compared to the strong correlation between clutch and egg load, host quality was found to be a weak factor in determining clutch size.

In an experiment which manipulated host plant quality, Rossi et al (1992) found that galls of *Asphondylia borrichiae* grew at a faster rate and to a larger size on *Borrichiae frutescens* plants with increased fertiliser treatment, and that there were more galls on these plants than on untreated ones. Rossi et al (1992) suggest that there may be an ovipositional preference for larger and more vigorous plants, associated with higher survival of offspring on these plants.

As mentioned in Chapter 4, some insects have been observed to use an Oviposition Deterring Pheromone, after ovipositing in hosts. The function of ODPs is to mark the host in which an insect has oviposited, so that this host will be more easily recognised by the same insect as it continues to forage for hosts (Averill and Prokopy, (1987). Once the ODP

strategy has become widespread in a population marking may also benefit other individuals which recognise the mark. It is assumed here that a single larva in a host has a better probability of survival than two or more larvae in hosts where super-oviposition has occurred.

6.1.4 Methodological problems

Many of the studies on the clutch size of insects have relied on the natural variation of clutch size for looking at the relationship between clutch size and fitness. The disadvantages of this method are:

1 That variation in the quality of hosts may be an important factor in larval mortality or in the reproductive success of offspring, and may therefore determine clutch size; whereas we really want to look at the effect of varying clutch size at constant host quality;

2 That the variation in clutch size is not sufficiently great in order to give data on maximum clutch size;

3 And that the sample sizes at low and high clutch sizes may be too small.

For some organisms, it is possible to manipulate clutch size, and to select hosts in such a way as to minimise variation in host quality. Such a method was used on the bethylid wasp *Goniozus* (Hardy et al 1992), which is a gregarious ectoparasitoid of small lepidoptera larvae. Here it was important to minimise variation in host size which influenced the fitness of clutches.

In the case of *Urophora jaceana* clutch size cannot be observed directly except by destructive dissection of flowerbuds. It was therefore necessary to consider larval survival in two developmental stages:

1 From oviposition to establishment of galls, when an indirect method was used to compare the frequency distribution of clutch size with the frequency distribution of the number of gall cells in flowerheads.

2 From establishment of galls up to the third instar stage, when the larvae are fully grown.

Section 2: Mortality of *Urophora jaceana* eggs and larvae before gall formation

In this section the aims are:

1 To determine whether or not there is significant mortality of gall fly eggs and first instar larvae up to gall formation.

2 To identify flowerheads in which more than one clutch has been laid.

3 To determine whether survival of eggs and larvae up to gall formation is a function of clutch size.

4 To estimate the constants in the survival curve for eggs and larvae up to gall formation, as a function of clutch size.

The method used is to compare the frequency distribution of the number of eggs oviposited in flowerbuds with the frequency distribution of the number of gall cells in flowerheads. The frequency distributions in both cases are constructed by dissecting flowerheads in censuses taken at the end of June for clutch size, and at the end of August for number of gall cells formed, whether or not they contained larvae.

6.2.1 Introduction

As Varley (1947) noted, the mortality of eggs and larvae up to the formation of galls cannot be obtained directly. However by obtaining a frequency distribution of the number of eggs laid in flowerheads in the field site, and comparing this distribution to the frequency distribution

of the number of gall cells found in flowerheads later in the season, it is possible indirectly to investigate mortality in the pre-gall formation stage.

In the article by Varley (1947), the difference between egg and gall frequencies was highly significant. This difference was attributed to mortality amongst the eggs and larvae prior to gall formation. In order to estimate the magnitude of this mortality, a transformation of egg frequencies was performed.

Varley assumed that a mortality factor, m, operated randomly on egg clutches, in such a way that the death of one egg or larva did not affect the survival of others in the same flowerhead. He argued that in a clutch of two eggs, there was a probability m^2 that both will die, a probability $2m(1-m)$ that only one would die, and a probability $(1-m)^2$ that neither would die. Thus the probabilities of the numbers of eggs and larvae dying in a clutch two eggs are in fact the terms of the binomial expansion. By induction, the terms of the binomial expansion can be used to generate the probable numbers of eggs and larvae dying in clutches of any size. Using the terms of binomial expansion [(1-m),m] for all clutch sizes, a transformed frequency distribution can therefore be produced. Thus the probabilities that one egg will survive are summed across the range of clutch sizes, to produce the transformed frequency of clutches of one egg after mortality. The probabilities that two eggs will survive are summed to produce the transformed frequency for two eggs, and so on up to the largest clutch size. Varley did not include frequencies of flowerheads with no eggs or galls in his tables, but the transformed frequency for flowerheads with no eggs after mortality is simply the sum of probabilities that no egg will survive plus the initial frequency of flowerheads with no eggs.

Varley (1947) found that the best estimate of m was 0.289, but noted that the assumption of random mortality was probably not correct, and that the mortality appeared to be higher for eggs in clutches of four or more, suggesting density dependance.

Using the frequencies of naturally occuring clutch sizes obtained in 1996, a similar transformation of clutch size frequencies was generated, first assuming, as Varley did, that the mortality factor, m, acts randomly. Then, with the advantage of computerisation, m as a function of clutch size is substituted for constant m.

6.2.2 Method

The method followed here depends on the fact that gall formation is induced by the second instar larva as it burrows into an individual floret of the flowerbud. Galls are not therefore present in the flowerbud at the egg stage, nor in the first few days after the second instar larva has emerged.

On 25th June and 30th June 1996, 234 flowerbuds of *Centaurea nigra* in the field site were marked with sticky paper labels, on the day when they became suitable for oviposition (Table 14). The labels were colour-coded, so that the day they were marked was easily recognised. Marked flowerheads were evenly spread over the whole of the field site. Marked flowerbuds were left for a period of six days on the plant after marking, to allow eggs to be laid in them, but not to hatch. They were then collected from the field and kept frozen so as to preserve any eggs that had been laid in them. 188 flowerheads were collected in total, some of the labels having been lost in the six day period.

Table 14: Schedule of marking and collecting flowerbuds

Date:		Number:		Number:
marked	collected	marked	collected	Without eggs
25th June	1st July	144	116	30
30th June	6th July	90	72	18

The flowerheads were dissected under X20 magnification, and the number of eggs discovered in each flowerhead was recorded. Some flowerheads contained more than one discrete clutch of eggs, differentiated by the fact that the eggs of each clutch were grouped together and occupied a different position to other groups within the flowerhead. When more than one clutch was identified in a flowerhead, the size of each clutch was recorded separately. On 26th August 1996, a sample of 244 flowerheads was collected from the field site. The flowerheads were allowed to dry indoors, and then were dissected, the contents of gall cells noted and also the number of gall cells per flowerhead.

6.2.3 Results

Of those flowerbuds which contained eggs, 22 contained two spatially separate clutches, which represents 15.7% of the sample (Table 15). Egg clutch sizes from one to fourteen were found in flowerheads. The largest clutch sizes appeared to be the result of two or more batches of eggs being oviposited in the same flowerhead (Table 15).

Table 15: Numbers of eggs in 22 pairs of clutches found in flowerheads.

Clutch 1	Clutch 2	Total
4	2	6
4	3	7
5	1	6
6	3	9
6	5	11
4	3	7
2	2	4
6	4	10
4	1	5
4	1	5
4	2	6
3	1	4
3	2	5
7	5	12
3	1	4
7	4	11
4	3	7
5	4	9
8	6	14
3	2	5
2	2	4
6	2	8

Table 16: The frequency of 1) clutch sizes and 2) number of gall cells in flowerheads sampled in 1996. The frequency of clutch sizes standardised to the same sample number of galls is also shown.

Clutch size	Frequency of clutch size	Frequency of clutch size (Standardised)	Number of gall cells per fl'head	Frequency of number of gall cells
0	48	62.3	0	83
1	20	26.0	1	22
2	23	29.9	2	35
3	35	45.4	3	33
4	25	32.5	4	28
5	13	16.9	5	22
6	10	13.0	6	10
7	5	6.5	7	5
8	2	2.6	8	2
9	2	2.6	9	3
10	1	1.3	10	1
11	2	2.6	11	0
12	1	1.3	12	0
13	0	0.0	13	0
14	1	1.3	14	0
Total:	188	244		244

The frequency distributions of number of eggs per flowerbud, and number of gall cells per flowerhead were calculated. The frequency of egg clutch sizes was standardised to the sample size of gall size distribution (Table 16). Clutch sizes of one occur frequently, suggesting that per capita survival decreases monotonically with clutch size (see Chapter 6, section 1, page 70).

The standardised frequencies were calculated as sample size of galls multiplied by the frequency of each clutch size divided by the sample size of clutches. As will be noted in Table 16, the number of flowerbuds containing zero eggs, and the number containing zero galls are included. The reason for this is that mortality of all eggs laid in flowerbuds will result in flowerheads containing zero gall cells. As it is impossible to distinguish between non-galled flowerheads in which no eggs were laid, and those in which all eggs have died, the difference between the frequency of flowerbuds with no eggs and those with no gall cells gives an indication of the number of flowerbuds in which there is total mortality of eggs.

A G test was then performed on the two frequency distributions. The standardised egg frequency was taken to be the expected distribution of gall cell sizes, assuming no mortality of eggs. Categories eight to fourteen were clumped to give a large enough sample size to perform the tests. The tests were performed on nine categories, and since the expected values were standardised using the sum of the frequencies of gall sizes, there were seven degrees of freedom. The value of G is 18.003, which is significant at $p < 0.025$.

The frequency distribution of eggs was transformed, following Varley's method of using a random mortality factor, m (Table 17). In order to find the value of m which generates the best expected transformed frequencies to fit the frequency of gall sizes, values of m between 0.11 and 0.16 were used in the binomial expansion. The value which gave the lowest value of G, is taken as the best estimate of mortality. The minimum G value was 14.13, occuring when m = 0.135, which is significant at $p < 0.05$, with seven degrees of freedom (Table 18).

The null hypothesis must be rejected, that there is no difference between the frequency distribution of eggs after it has been transformed by applying a random mortality factor and the expected frequency of galls. The assumption that random mortality acts independantly of clutch size therefore does not hold.

Table 17: Transformation of egg frequency using Varley's method. Mortality factor, m, constant at 0.135.

The columns headed 0 to >6 are the terms of the binomial expansion corresponding to the probabilities that 0,1,2.....14 eggs will die in a particular clutch. The rows give the binomial terms which summed give the transformed frequencies. The diagonals from bottom to top, left to right, are the binomial terms for each clutch size, which added together equals the original frequency. For example, the highlighted numbers represent the binomial expansion of the probability of n larvae dying in an original clutch size of 7, and clutch size frequency of 6.5.

Table 17: contd....

Clutch size	Clutch size frequency	Transformed clutch size frequency	Mortality	Number of clutches in which n larvae die							
				n = 0	1	2	3	4	5	6	>6
0	62.3	66.5	0.135	0	3.5	0.54	.11	.01	0	0	0
1	26.0	31.9	0.135	22.45	6.97	2.15	.28	.02	0	0	0
2	29.9	39.1	0.135	22.34	13.77	2.65	.31	.05	0	0	0
3	45.4	43.2	0.135	29.40	11.34	1.99	.41	.05	0	0	0
4	32.5	26.9	0.135	18.17	6.38	1.99	.31	.03	.01	0	0
5	16.9	14.7	0.135	8.17	5.09	1.20	.17	.05	.01	0	0
6	13.0	8.8	0.135	5.44	2.57	0.55	.22	.03	.03	0	0
7	6.5	4.2	0.135	2.35	1.02	0.62	.11	.12	.01	0	0
8	2.6	2.5	0.135	0.81	0.99	0.26	.37	.05	.00	.01	
9	2.6	2.0	0.135	0.70	0.37	0.79	.15	.00	.02		
10	1.3	1.6	0.135	0.23	1.01	0.28	.00	.08			
11	2.6	0.9	0.135	0.41	0.33	0.00	.08				
12	1.3	0.5	0.135	0.18	0.00	0.29					
13	0.0	0.3	0.135	0.00	0.29						
14	1.3	0.1	0.135	0.13							

Table 18: Values of G, derived by fitting the transformed clutch size frequencies to frequency of number of gall cells per flowerhead, for values of the mortality factor, m, between 0.11 and 0.16.

Mortality factor, m	G	df	Significance
0.11	15.04	6	$p < 0.05$
0.12	14.42	6	$p < 0.05$
0.13	14.16	6	$p < 0.05$
0.135	14.13	6	$p < 0.05$
0.14	14.16	6	$p < 0.05$
0.15	14.37	6	$p < 0.05$
0.16	14.75	6	$p < 0.05$

In order to investigate further the nature of the mortality factor, a different assumption was tested, that the survival of eggs and larvae up to gall formation is described by a quadratic function, of the form:

$$\text{Number of larvae surviving, } S = a + bN + cN^2 \qquad 1$$

where a,b,c are constants, and N is the clutch size. The mortality factor, m, can then be determined as the per capita mortality of larvae. The equation then becomes:

per capita mortality, m = 1 - number of larvae surviving/ clutch size

or $$m = 1 - (a/N + b + cN) \qquad 2$$

Equation 1 has one maximum value when the differential is zero. So that

$$dN/dS = b + 2cN^* = 0 \qquad 3$$

where N* is the clutch size when the number surviving is maximum. Therefore:

$$c = -b/2N^* \qquad 4$$

Furthermore, when N = 1,

$$a = (1-m) - b - c \qquad 5$$

Table 19 is set out in the same way as Table 17, except the values of m have been generated from equation 2, using estimated values for a, b and c. In this case a = -0.0396, b = 1.02 and c = -0.0204, and N* = 25.

In a G test comparing the transformed clutch size frequency distribution shown in Table 19 with the gall size frequency distribution, the value of G is 13.70, which is less than the critical value of 14.067 with seven degrees of freedom at p = 0.05. There is no reason then to reject the null hypothesis that there is no significant difference between the transformed clutch size frequency distribution and the gall size frequency distribution. The assumption that the function describing the survival of eggs and larvae up to gall formation is a quadratic function of clutch size is supported by this result.

Table 19: Transformation of egg frequency using Varley's method. Mortality factor, m, generated by equation 1, with a = -0.0396, b = 1.02 and c = -0.0204. For explanation of the table, see legend to Table 4.

Clutch size	Clutch size frequency	Transformed clutch size frequency	Mortality	Number of clutches in which n larvae die							
				n = 0	1	2	3	4	5	6	>6
0	62.3	63.4		0	1.04	0.05	.01	.00	0	0	0
1	26.0	27.7	0.04	24.92	2.33	0.38	.04	.01	0	0	0
2	29.9	35.1	0.04	27.48	6.63	0.86	.10	.02	0	0	0
3	45.4	47.1	0.05	38.41	7.43	1.03	.24	.04	.01	0	0
4	32.5	31.1	0.07	24.12	5.20	1.46	.28	.05	.02	0	0
5	16.9	16.8	0.09	10.53	4.77	1.13	.21	.10	.02	.02	0
6	13.0	10.2	0.11	6.49	2.56	0.61	.34	.06	.06	.02	0
7	6.5	4.6	0.13	2.48	1.00	0.73	.15	.17	.06	.00	0
8	2.6	2.4	0.15	0.72	0.90	0.24	.33	.13	.00	.09	
9	2.6	1.5	0.17	0.50	0.23	0.42	.20	.00	.17		
10	1.3	0.9	0.19	0.10	0.32	0.20	.00	.23			
11	2.6	0.5	0.21	0.12	0.13	0.00	.23				
12	1.3	0.2	0.23	0.04	0.00	0.15					
13	0.0	0.1	0.25	0.00	0.06						
14	1.3	0.0	0.27	0.01							

Table 20: Values of the test statistic, G, derived by fitting the transformed clutch size frequencies generated by mortality, m = 1 - (a/N + b + cN) (equation 2) to frequency of number of gall cells per flowerhead, for different parameter values.

Clutch size* = clutch size at which per capita mortality is minimum. N* = Lack Solution clutch size. ns not sig.; * sig. at P < 0.05.

For clutch size* = 1, G is minimum (= 13.70) when N* = 25, a = -0.04, b = 1.02, c = -0.02.

Parameters: C = -0.02		G N* = 24	Clutch size*	G N* = 25	Clutch size*	G N* = 26	Clutch size*
a	b						
-0.04	1.02	13.74ns	1	13.70ns	1	13.71ns	1
-0.05	1.03	13.57ns	2	13.58ns	2	13.63ns	2
-0.06	1.04	13.47ns	2	13.53ns	2	13.63ns	2
-0.03	1.02	13.96ns	1	13.93ns	1	13.94ns	1
-0.04	1.03	13.81ns	1	13.83ns	1	13.88ns	2
-0.05	1.04	13.72ns	2	13.79ns	2	13.89ns	2
-0.02	1.02	14.19*	1	14.17*	1	14.19*	1
-0.03	1.03	14.05ns	1	14.08*	1	14.14*	1
-0.04	1.04	13.98ns	1	14.05ns	1	14.17ns	1/2
-0.01	1.02	14.42*	1	14.41*	1	14.44*	1
-0.02	1.03	14.30*	1	14.33*	1	14.41*	1
-0.03	1.04	14.24*	1	14.33*	1	14.45*	1

The set of parameters of the quadratic equations 1 and 2, used in Table 19, is just one possibility among many sets which will produce a non-significant value of G. However, a best esimate can be arrived at by using different sets of the parameters in turn to generate the series of m values, and fitting the resulting transformation of clutch size frequency distributions against the frequency distribution of gall cell numbers, using the G test. The lowest value of G is produced by the best fitting set of parameters. One further parameter was calculated for each set, the clutch size at which the mortality factor is at its minimum value (Table 20). The set which gives the lowest value of G and a clutch size of one at which the mortality factor is at its minimum, is that used in Table 19.

There is no reason then to reject the the null hypothesis that there is no difference between the frequency distribution of eggs transformed by the terms of the binomial expansion of [(1-m),m], with m generated by equation 2, and the expected frequency distribution of galls.

6.2.4 Discussion

The assumption that mortality acts randomly, and is not dependant on clutch size, was found not to hold. Deploying a range of values for random mortality, held constant over the range of clutch sizes, produced a transformed frequency of clutch sizes which was significantly different to the frequency of gall sizes. This conclusion concurs with Varley's assessment of his data.

The alternative assumption, that mortality is dependant on clutch size, and specifically that the survival of larvae up to gall formation is a quadratic function of clutch size, is tentatively supported by these data. Although the quadratic equation, which generates a transformed frequency of egg clutch size, which fits best with the frequency of gall size, and which gives a minimum mortality factor at clutch size one, brings the value of G below the level of significance at $p = 0.05$, the margin is not very great, and it is not known whether the quadratic coefficient is significantly greater than zero. It would therefore be desirable to repeat this field work with larger sample sizes, and to obtain standard errors of the coefficients by boot-strapping.

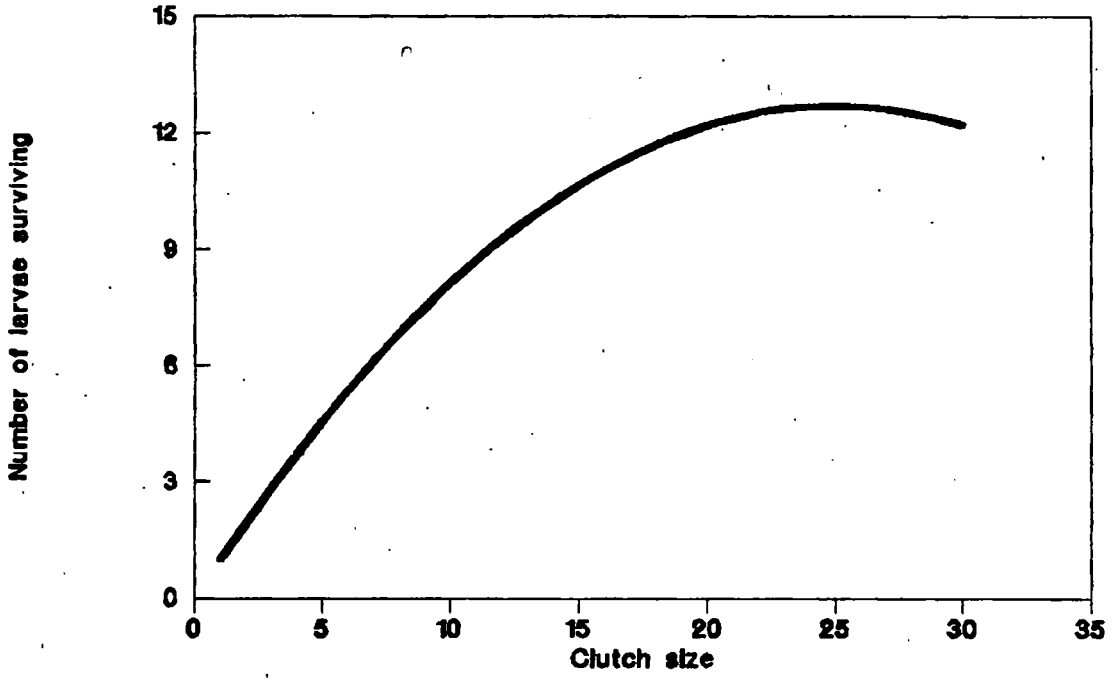
The parameters of the best fitting equation are:

$$S = -0.0396 + 1.02N - 0.0204N^2.$$

The survival curve given by this equation (Figure 5) is convex, reaching a maximum at clutch size 25. The corresponding per capita survival graph (Figure 6) reveals that per capita survival decreases with increased clutch size, from its maximum at clutch size one. Per capita survival in the egg to gall formation stage as described by the equation therefore decreases monotonically with clutch size. This is an important feature of the equation since in the trade-off between present gain in fitness and expected future gains, clutch sizes of one are possible.

Figure 5: Number of larvae per clutch surviving up to gall formation plotted against clutch size.

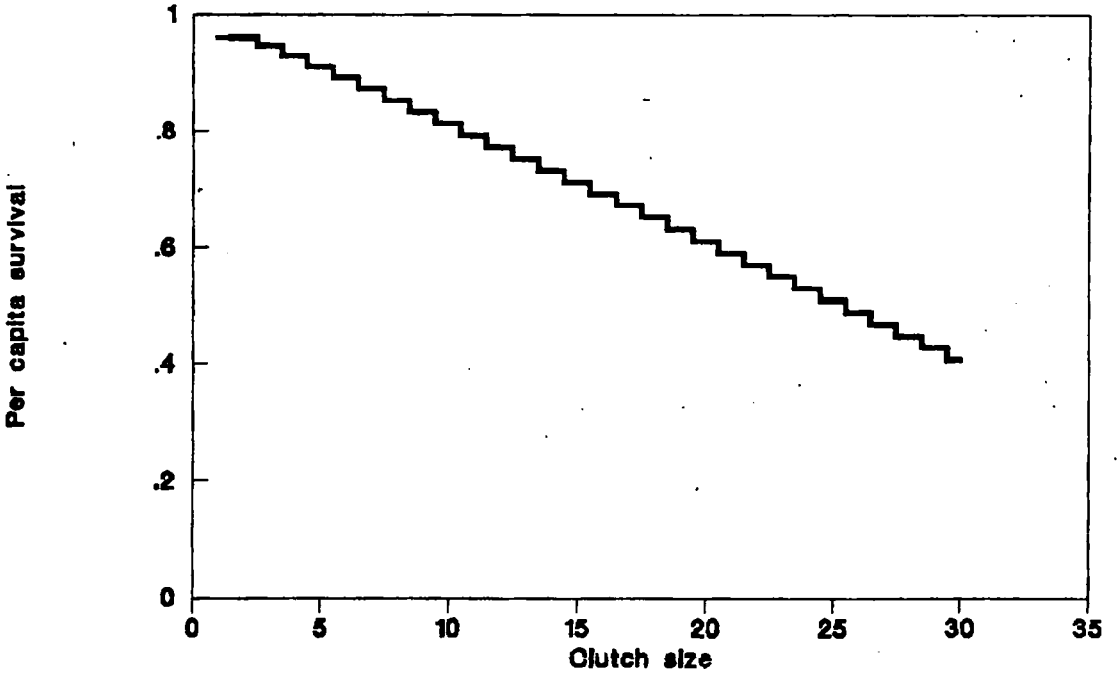
Clutch size survival function:
S = -0.0396 + 1.02.C - 0.0204.C.C



Maximum Clutch Size = 25

Figure 6:
Per capita survival per clutch as a function of clutch size:

Clutch size survival function:
S = -0.0396 + 1.02.C - 0.0204.C.C



Per capita survival decreases monotonically at clutch sizes > 1

Wilson and Lessells (1994) identify larval competition as a major mortality factor and Godfray (1987) lists some studies in which it is a significant mortality factor, including *Nasonia vitripennis* (Charnov and Skinner 1984, 1985), and six species of parasitic wasps (Waage and Godfray 1985).

In the case of gall fly eggs and larvae, the causes of mortality prior to gall formation are not observable, although on one occasion Varley (1947) found two larvae in the same floret within a flowerbud. He suggested that competition between larvae may be one cause of mortality, and would explain the increased mortality at larger clutch sizes. Larval competition may also lead to reduced larval size. If this is the case, investigating the variation of larval size against larval density would be a direct way of testing whether larval competition occurs.

Section 3: The survival of gall fly larvae from gall formation up to the third instar

In this section the factors of mortality and the survival of gall fly larvae from the time of gall formation up to the third instar are investigated in order to provide further parameter estimates for the dynamic model. The aims of this section are thus:

1 To identify any patterns of gall fly larval survival in relation to gall size.

2 To identify and analyse those factors which contribute to the mortality of gall fly larvae in relation to gall size.

3 To estimate the parameters of survival of larvae as a function of gall cell number.

The natural variation of the number of gall cells found in individual flowerheads is used to determine the survival of larvae of *Urophora jaceana* in relation to gall size. By dissecting galls collected from the field and identifying gall cell contents, the magnitudes of mortality factors and of survival over the range of gall cell numbers were identified.

6.3.1 Introduction

The previous section of this Chapter considered the survival of *Urophora jaceana* eggs and larvae up to the establishment of galls. In this section, the survival of larvae from gall formation up to the fully-grown third instar stage is investigated.

The number of gall cells in flowerheads of *Centaurea nigra* varied between one and sixteen within the field study site. Part of this variation may be explained in terms of super-oviposition, which has been observed directly in the field (Chapter 4) and in experimental conditions (Chapter 8, Varley 1947); part by egg mortality (Chapter 6), and part by variation in clutch size (Chapter 6). Direct observation of newly-oviposited eggs suggests that they are laid in clutches of between one and six or seven eggs (Chapter 8).

Godfray (1987) summarises the mechanisms by which clutch size may influence the fitness of individuals within the same clutch. For gall fly larvae in multiple gall cells, it is possible that competition between larvae for plant resources, and predation and parasitism of larvae are mechanisms which may be related to clutch size.

A number of agents are responsible for the mortality of larvae within galls. Attack by parasitoids (Chapter 2, Varley 1947, Dempster et al 1995a) occurs frequently. In the field site, four parasitoids have been identified. *Eurytoma curta* is the most common, and is the only one that is truly specific to *Urophora jaceana* (Varley 1935,1947, Dempster et al 1995b). *Eurytoma curta* attacks larvae before the gall tissue is hardened and before the sclerotised foot of the larvae effectively closes the gall cell. The time period in which attack occurs is in the first two weeks after gall formation (Varley 1935,1947). The other three, *Cyranimus torymus*, *Macroneura vesicula*, and *Tetrastichus spp B* are known to super-parasitise, and will attack larvae of *Eurytoma curta* as well as of *Urophora jaceana*.

Predation of gall fly larvae by lepidopteran larvae has also been identified in the field site, and the moths *Eucosma hohenwartiana* and *Euxanthis straminea* (Varley 1947) have been identified. The lepidopteran larvae eat flowerhead contents indiscriminately, including seeds, receptacle and gall tissue, and the contents of galls, including gall fly and other larvae.

In addition to these two categories of mortality, dead gall fly larvae are sometimes found in gall cells. The causes of this mortality are not known, but may be associated on the one hand with viral attack, or with competition for resources.

Competition for resources is probably not an important cause of mortality in the larval stage. It is possible, however, that adult gall flies, emerging from large clutches are smaller, die sooner, and produce fewer offspring than those in smaller clutches.

Utilising the natural variation of the number of gall cells per flowerhead, the question to be investigated here is whether any of these mortality factors act in any way which is dependent on the number of gall cells present in a flowerhead.

6.3.2 Method

In 1995, 126 gall-bearing flowerheads of *Centaurea nigra* were selected from the field study site in October. The sampling attempted to insure that as wide as possible a range of gall cell number per flowerhead was selected.

The galls were dissected, and the contents and number of gall cells were noted. The numbers of surviving gall fly larvae, of dead gall fly larvae, of larvae of the parasitoids *Eurytoma curta* and *Torymus cyranimus*, and of gall cells which were empty or contained lepidopteran larval frass, were recorded for each gall complex, as well as the total number of gall cells. In addition, the presence of lepidopteran larvae was also noted.

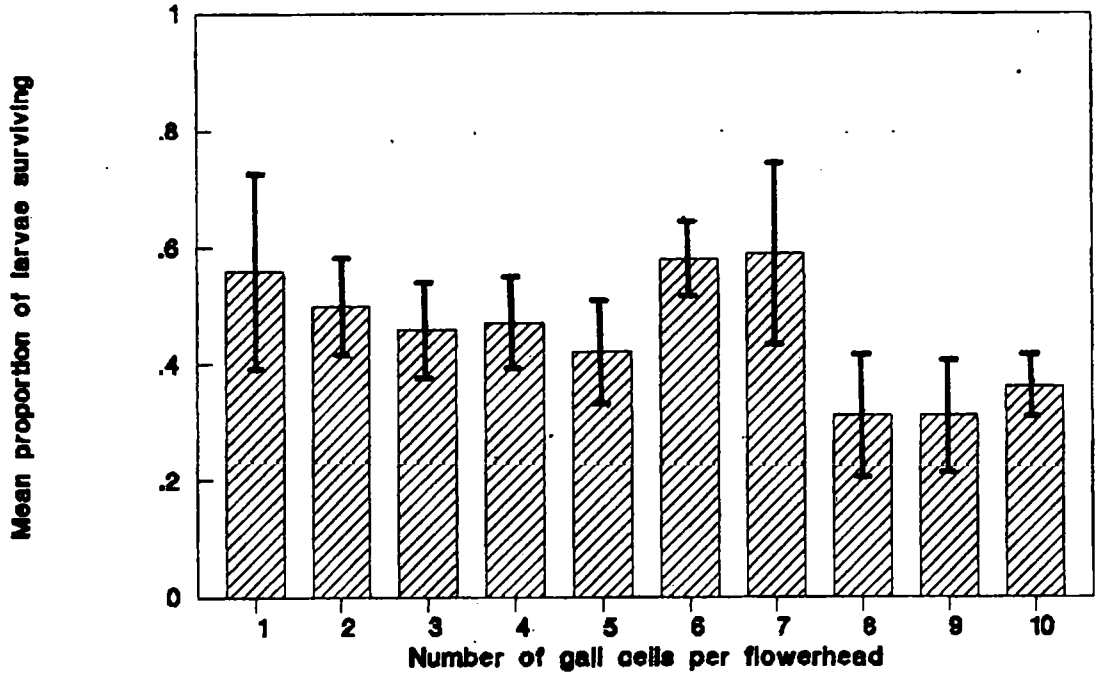
6.3.3 Results

126 gall bearing flowerheads were dissected. Table 21 gives the frequencies of each gall size category for the 126 flowerheads in the sample.

The values of interest here are the proportion of gall cells from each flowerhead which contain the three categories of cell contents. In other words, the proportion of cells attacked by *Eurytoma curta*, the proportion which are empty, and the proportion in which there are live gall fly larvae. The statistical programme GLIM is used, with the binomial error structure and the identity link (Crawley 1992 chapter 13). This model transforms the proportional data into logits, which is the natural logarithm of the ratio of the probability of the event happening to the probability of it not happening. The programme also weights the unequal sample sizes.

Figure 7 shows the mean proportion of gall fly larvae surviving plotted against the gall size category. The mean proportion in categories one to seven appears to be more than in categories eight to ten, a pattern which is investigated in the following tests.

Figure 7:
Mean proportion of gall fly larvae surviving up to third instar plotted against number of gall cells per flowerhead.



6.3.3.1 The proportion of gall cells which are empty

The reduction in deviance (see Chapter 2, section 4, page 27) as a result of fitting the proportion of gall cells which are empty against the gall size categories is significant at $p < 0.01$ (Table 22), suggesting that the proportion of cells which are empty increases with the number of gall cells in the flowerhead.

Combining gall size categories one to seven, and eight to ten, did not increase the residual deviance significantly, when compared to the model including all ten categories separately (Table 22), and therefore does not reduce the explanatory power of the model. The mean proportion of empty gall cells per flowerhead is therefore significantly less (0.27 +-

0.032) in flowerheads containing seven or less gall cells compared to the mean proportion of gall cells in flowerheads containing eight or more gall cells (0.51 +- 0.040) (Table 22).

The proportion of flowerheads which contain empty gall cells was tested against gall size category (Table 23). A X^2 test shows that there is a significantly lower proportion of flowerheads which contain empty gall cells in smaller gall size categories than in larger categories at $p < 0.005$ (Table 23).

Table 21: The frequencies of gall size categories in the sample of 126 flowerheads. Category 10 combines flowerheads with between 10 and 16 gall cells.

Gall size category	Number of gall cells per flowerhead	Number in sample
1	1	9
2	2	26
3	3	18
4	4	19
5	5	12
6	6	11
7	7	7
8	8	6
9	9	6
10	10 - 16	12

Table 22: Analysis of deviance table: The proportion of empty gall cells per flowerhead fitted against the gall cell categories after correcting for overdispersion using Pearson's X^2 to calculate the scale parameter.

When categories 1 to 7 and 8 to 10 are combined, and overdispersion corrected for, there is a non significant increase in scaled deviance of 3.97 with a decrease in degrees of freedom of 8 (F ratio = 0.50), compared to the full model. Combining categories 1 to 7 and 8 to 10 does not therefore reduce significantly the explanatory power of the model.

All ten gall cell categories included.

Source of deviance:	Scaled Deviance	d.f	F ratio
Among gall cell categories	23.52	9	2.61 **
Within gall cell categories	128.89	116	
Total	152.41	125	
Scale parameter 1.644			

Categories 1 to 7 and 8 to 10 combined:

Among gall cell categories	19.55	1	19.55 **
Within gall cell categories	144.15	124	
Total	163.70	125	
Scale parameter 1.530			

Table 22 contd....

Mean and variance of proportion of empty gall cells per flowerhead in combined categories 1 to 7 and 8 to 10.

Gall size category	Mean proportion empty	variance	Sample no.
<=7	0.27	0.104	102
> 7	0.51	0.038	24

Table 23: Chi square test on proportion of flowerheads attacked by lepidoptera in categories of gall cells per flowerhead. Figures in brackets are the expected values if there is no difference between gall size categories in rate of attack.

Number of gall cells:	>=8	4,5,6,7	<=3	Total
Flowerheads attacked	24 (15.2)	36 (31.1)	20 (33.7)	80
Flowerheads not attacked	0 (8.8)	13 (17.9)	33 (19.3)	46
Total	24	49	53	126

Chi square = 31.07 significant at $p < 0.005$.

6.3.3.2 The proportion of gall cells attacked by *Eurytoma curta*

The proportion of gall cells attacked by Eurytoma curta per flowerhead is not significantly dependent on the number of gall cells in the flowerhead at $p = 0.05$, when over-dispersion is taken into account (Table 24).

Table 24: Analysis of deviance table: The proportion of gall cells attacked by *Eurytoma curta* per flowerhead fitted against the gall size categories, after correcting for overdispersion. Categories 1 to 9 correspond to 1 to 9 gall cells per flowerhead respectively. Category 10 combines 10 to 16 gall cells per flowerhead. When categories 1 to 7 and 8 to 10 are combined, there is a significant increase in residual deviance of 17.49. * $p < 0.05$

All 10 categories fitted:

Source of deviance:	Deviance	d.f.	F ratio
Among gall cell categories	8.99	9	1.00 ns
Within gall cell categories	104.38	116	
Total	113.37	125	
Scale parameter 1.94			

6.3.3.3 The proportion of cells attacked by *Eurytoma curta* plus empty cells.

When the proportion of cells attacked by *Eurytoma curta* is combined with the proportion of empty gall cells, the proportion increases significantly with the gall size category at $p < 0.05$, after correcting for overdispersion (Table 25). Combining gall size categories one to seven and eight to ten results in an increase of residual scaled deviance of 3.80, with a decrease of eight degrees of freedom (F ratio = 0.48), which is not significant, and does not reduce the explanatory power of the model.

Table 25: Analysis of deviance table: The proportion of gall cells attacked plus empty gall cells per flowerhead fitted against the gall cell categories after correcting for overdispersion.

When categories 1 to 7 and 8 to 10 are combined, and overdispersion corrected for, there is a non significant increase in scaled deviance of 3.97 with a decrease in degrees of freedom of 8 (F ratio = 0.48), compared to the full model.

All ten gall cell categories included.			
Source of deviance:	Scaled Deviance	d.f	F ratio
Among gall cell categories	20.14	9	2.24 *
Within gall cell categories	141.35	116	
Total	161.49	125	
Scale parameter 1.913			
Categories 1 to 7 and 8 to 10 combined:			
Among gall cell categories	16.34	1	16.34 **
Within gall cell categories	154.59	124	
Total	170.92	125	
Scale parameter 1.808			

6.3.3.4 Proportion of cells attacked by *Torymus cyranimus* plus cells with dead larvae

The proportion of cells attacked by *Torymus cyranimus* plus cells with dead larvae was found to decrease significantly with increasing gall size category at $p < 0.001$ (Table 26). Combining gall size categories resulted in significant increase of residual deviance.

6.3.3.5 Proportion of gall fly larvae surviving

The proportion of gall fly larvae surviving is significantly higher in gall size categories one to seven (mean proportion = 0.50 +– 0.036) than in categories eight to ten (mean proportion = 0.34 +–0.045) at $p < 0.01$ (Table 27).

Table 26: Analysis of deviance table: The proportion of gall cells attacked by *Torymus cyranimus* plus cells with dead larvae per flowerhead fitted against the gall size categories. There was only a small amount of overdispersion. Combining gall size categories resulted in a significant increase of deviance of 21.70 with a decrease in degrees of freedom of eight. *** $p < 0.001$

All 10 categories fitted:

Source of deviance:	Deviance	d.f.	X^2
Among gall cell categories	31.39	9	31.79 ***
Within gall cell categories	148.16	116	
Total	179.55	125	

Table 27: Analysis of deviance table: The proportion of *Urophora* larvae surviving per flowerhead was fitted against gall cell categories one to seven and eight to ten, after correcting for overdispersion. ** p < 0.01.

All ten gall cell categories included.

Source of deviance:	Scaled Deviance	d.f	F ratio
Among gall cell categories	8.94	1	8.94**
Within gall cell categories	156.08	116	
Total	165.02	125	

Mean and variance of proportion of *Urophora jaceana* larvae surviving per flowerhead in combined categories 1 to 7 and 8 to 10.

Gall size category	Mean survival	Variance	Sample no.
<=7	0.50	0.134	102
> 7	0.34	0.049	24

6.3.4 Discussion

The proportion of gall cells which are empty is greater in gall cell categories eight and over than in categories seven and less (Table 22). Although the proportion of gall cells attacked by *Eurytoma curta* is not dependent on gall size category (Table 24), the combination of empty gall cells and attack by *Eurytoma curta* remains significantly higher in

gall cell categories of eight and over (Table 25). The other mortality factors combined (attack by *Torymus cyranimus* and dead larvae) decrease with increasing gall size category (Table 26).

The combination of these factors results in flowerheads with seven cells and less having a significantly higher proportion of larvae surviving than in flowerheads with eight or more gall cells (Table 27).

Can this analysis be interpreted biologically? Empty gall cells appear to be the work of generalist lepidopteran larvae (Varley 1947, Dempster et al 1995a), with frass often found in empty cells (personal observation). If this is the case, the proportion of larvae eaten by lepidoptera is significantly higher in flowerheads with eight or more gall cells, than in flowerheads with seven or less gall cells. Furthermore, the rate of attack by lepidopteran larvae is higher the larger is the gall size category (Table 23).

This result suggests that lepidopteran larvae show a preference for flowerheads with larger numbers of gall cells. It is possible that the preference is for the succulent gall tissue, the amount of which in a flowerhead is directly related to the number of gall cells present. Such a preference would make larvae in larger groups of gall cells more vulnerable to predation.

The effects of other mortality factors appear to weaken the density dependence of lepidopteran predation, which makes this analysis more complicated.

It has been noted (Chapter 5) that the number of eggs laid at each oviposition is in the range of one to seven. Larger clutches are then the result of super-oviposition (Chapter 6). Correspondingly, galls with more than six or seven gall cells are those which have formed from eggs laid in more than one clutch. Ovipositing a second clutch in a flowerhead, thereby increasing the number of gall cells above the critical number for significantly increased predation by lepidoptera, may then result in a lower per capita return for both the second clutch and also for the first clutch.

Chapter 7: A Dynamic Model for *Urophora jaceana* ovipositing in flowerheads of *Centaurea nigra*

Section 1: Developing the Model

The aims of this section are to:

1 Establish the most realistic dynamic modelling equation for the oviposition behaviour of *Urophora jaceana*, by applying results of clutch size and gall size analysis.

2 Develop a computer programme, with the dynamic modelling equation at its heart, which may be used to make predictions concerning oviposition behaviour of gall flies in different circumstances.

3 To include realistic parameters of search and oviposition behaviour in the computer model, which then may be used to make predictions of population level phenomena.

7.1.1: The dynamic modelling equation

The dynamic modelling equation to be used here is that proposed by Mangel and Clark (1988, chapter 4, equations 4.10, 4.17), for an insect with mature eggs only, ovipositing in non-parasitised and parasitised hosts (Table 28). The state variables incorporated into the Dynamic Modelling Equation of Table 28, are the egg load of the fly (X(t)), and the number of flower buds available for oviposition ($N_1(t)$ and $N_2(t)$).

Table 28: The Dynamic Modelling Equation, from Mangel and Clark (1988), with explanation of the parameters, variables and functions which are incorporated into the equation.

Equations

$$F(n,x,t,T) = l_0(t).p_0(t).F(n,x,t+1,T) + l_1(t).\underset{0<=c<=8}{MAX}\{W_1(c,x,t) + p(c,x,t).F(n+1,x-c,t+1,T)\} + l_2(t).\underset{0<=c<=8}{MAX}\{W_2(c,x,t) + p(c,x,t).F(n,x-c,t+1,T)\} \quad 1$$

where

$$l_0(t) = \exp(-eN_0) \quad 2.1$$

$$l_1(t) = ((1-\exp(-eN_0))N_1(t)/N_0) \quad 2.2$$

$$l_2(t) = ((1-\exp(-eN_0))N_2(t)/N_0) \quad 2.3$$

$$N_0 = N_1(t) + N_2(t) \quad 2.4$$

and

$$p(c,x,t) = 1 \quad \text{for } t < T \quad 3.1$$

$$p(c,x,t) = 0 \quad \text{for } t = T \quad 3.2$$

and

$$W_1(c,x,t) = 0.5(-0.0396 + 1.02c_1 - 0.0204c_1^2) \quad 4.1$$

$$W_2(c,x,t) = 0.34(-0.0396 + 1.102(8+c_2) - 0.0204(8+c_2)^2)c_2/(8+c_2) \quad 4.2$$

Parameters of equations		Value	
T	The final time interval of the gall fly's life	75	(estimate)
	Length of time interval	15 mins	(Ch 4)
X(0)	Initial egg load of fly	70	(estimate)
e	Random search coefficient	0.004	(Ch 4)

State variables		Range
X(t)	Egg load in time interval t	0 to 70
	Initial egg load	70
N_0	Number of flower buds available for oviposition	0 to 300
$N_1(t)$	Number of non-parasitised flower buds in time interval t	0 to 300
$N_2(t)$	Number of parasitised flower buds in time interval t	0 to 300

Variables in equation 1

t	The present time interval	of 15 minutes
n	The number of parasitised flowerbuds in the search area	$N_2(t)$ $N_1(t) = N_0 - n$
x	The number of eggs available for oviposition (egg load)	X(t)
c	The clutch size	0 to 8 (Ch 6)

Functions included in equations 1, 2, 3, 4

F(n,x,t,T)	The maximum expected lifetime fitness obtained by oviposition, between time interval t and the final interval T, with eggload x and number of parasitised hosts n (Equation 1).
F(n+1,x-c, t+1,T)	The maximum expected lifetime fitness obtained by oviposition, between the time interval following t and the final interval T, having oviposited a clutch of size c in a non-parasitised flower bud in time interval t (Equation 1).

$l_0(t)$ The probability of the gall fly not encountering a flower bud in time interval t (Equation 2.1).

$l_1(t)$ The probability of the gall fly encountering a non-parasitised flower bud in time interval t (Equation 2.2).

$l_2(t)$ The probability of the gall encountering a parasitised flower bud in time interval t (Equation 2.3).

$p(c,x,t)$ The probability that the gall fly will survive time interval t, with egg load x, and ovipositing a clutch of size c (Equations 3.1 and 3.2).

$W_1(c,x,t)$ The fitness gain by ovipositing a clutch of size c in a non-parasitised flower bud, at egg load x, in time period t (Equation 4.1).

$W_2(c,x,t)$ The fitness gain by ovipositing a clutch of size c in a parasitised flower bud, at egg load x, in time period t (Equation 4.2).

The reasoning behind equation 2.4 is that each time oviposition occurs in a non-parasitised flower bud, the number of such flower buds available for future oviposition decreases by one, and the number of parasitised flower buds increases by one. Super-oviposition is assumed not to affect the number of parasitised flower buds. Likewise, if a clutch of size c is oviposited in one time interval, then X(t)-c eggs remain for the next time interval.

As will be appreciated from the Dynamic Modelling Equation the potential number of variables, and the range of each variable is very great. In order to save computing time, a number of assumptions have been made in setting the parameters of the model, particularly in setting the number of time intervals and the initial egg load.

Number of time intervals

In Chapter 4, the shortest time interval in which a female gall fly may search for an oviposition site and oviposit was found to be approximately 15 minutes. The number of time intervals of 15 minutes between the first and final intervals has been set at 75. Varley (1947) noted that the average lifespan of flies in the field is about eight days, although this is a very rough estimate. In addition he suggests that oviposition begins three days after eclosion. Flies may then be actively searching and ovipositing on average for a period of about five days. In Chapter 6 it was found that flies searched and oviposited for approximately 60% of its active time, which may amount to six hours per day, or 24 time intervals of 15 minutes.

In terms of the requirements of the model, a compromise had to be reached between a realistic number of time intervals in which flies could search, and the computing time needed to run the model. The model was found to reach stationarity for a given number of available flowerbuds at $t < 75$. Stationarity is when the outcome, in terms of clutch size and super-oviposition choices, of the Dynamic Modelling Equation does not change beyond a certain number of time intervals before the final interval. Although 75 active time intervals of fifteen minutes represents

a shorter lifespan than found in the field, three days instead of five, it is sufficiently long not to bias seriously the oviposition choices in the model.

Initial egg load

My own dissection of flies suggests that egg loads lie in the range of between 50 and 150. Varley (1947) suggested that the fecundity of gall flies in the field in 1935 was 70, and in 1936, 45 eggs. However, in experimental conditions, the mean fecundity for unfed flies was 95. Again, a compromise between an accurate initial egg load, and the practical constraints of the model had to be made. Therefore, an initial egg load of 70 eggs was used, which may be lower than suggested by the evidence.

An assumption was also made concerning the ovarian dynamics of the gall fly, that it is pro-ovigenic. In other words, the full complement of eggs is produced before oviposition begins, and that no more eggs develop once oviposition begins. The fact that flies are not observed to feed in the field (Chapter 4) supports this assumption, although it is possible that new egg production could be fuelled from fat reserves. Flies do not begin to oviposit until three days after eclosion (Varley 1947), suggesting that this period is important for the maturing of eggs. A similar assumption is made for Mangel's Model 1 (Mangel 1987), which he suggests is not too unrealistic for Lepidoptera, for some Coleoptera and particularly for some Diptera. For example in the models developed for *Rhagoletis*, pro-ovigenicity is assumed (Roitberg and Mangel 1988).

The survival function, p(c,x,t)

An assumption was made concerning the probability of a gall fly surviving a given time interval, p(c,x,t), which simplifies the equation, and reduces the number of state variables. The probability of surviving a time interval was counted as one in all time intervals before the final interval, and as zero in the final interval, as Mangel and Clark discuss (1988 chapter 4).

The fitness gain function

In Chapter 6, it was established that clutch size varies, and that the mortality of eggs and larvae up to gall formation is not random, but can be described as a function of clutch size. The following quadratic function was found to generate a distribution of gall sizes from an initial distribution of clutch sizes, which did not differ significantly from the observed distribution of gall sizes, and which allowed clutch size one to have maximum per capita survival,

$$Sc = -0.0396 + 1.02C - 0.0204C^2 \qquad 5$$

where Sc is the number of eggs surviving to gall formation in a clutch of size C. Sc is maximum at C = 25.

Also in Chapter 6, it was established that there was a significant difference in the proportion of larvae surviving from gall formation up to third instar between gall sizes up to seven galls, and gall sizes over

seven galls. For gall sizes up to seven galls, the proportion of gall fly larvae surviving was 0.5, and for gall sizes greater than seven the proportion was 0.34.

Super-oviposition was also observed in the field, and on dissection of flowerbuds, more than one clutch of eggs was discovered in 22 cases out of 188.

In the light of these results, the most realistic dynamic modelling equation should therefore combine clutch size and super-oviposition choices, which the functions $W_1(c,x,t)$ and $W_2(c,x,t)$ in Table 1 do. these equations are derived as follows.

Combining the clutch size survival with the differential survival of larvae up to third instar, the following two equations describe approximately survival from egg to third instar:

$$\text{If } C <= 8 \quad \text{then} \quad W = 0.5(-0.0396 + 1.02C - 0.0204C^2) \qquad 6.1$$

$$\text{If } C > 8 \quad \text{then} \quad W = 0.34(-0.0396 + 1.02C - 0.0204C^2) \qquad 6.2$$

where W is the number of larvae surviving in a clutch size of C from egg to third instar.

These equations would produce clutch sizes no greater than eight unless super-oviposition is to be incorporated, because of the large per capita decrease in survival between clutch size eight and nine. If the first clutch oviposited in a flowerhead is C_1 and a subsequent super-oviposited clutch is C_2, then:

$$W_1(C) = 0.5(-0.0396 + 1.02C_1 - 0.0204C_1^2) \quad 6.2$$

and

$$W_2(C) = 0.5(-0.0396 + 1.02(C_1+C_2) - 0.0204(C_1+C_2)^2)C_2/(C_1+C_2) \quad 6.3$$

if $(C_1 + C_2) <= 8$, or:

$$W_2(C) = 0.34(-0.0396 + 1.02(C_1+C_2) - 0.0204(C_1+C_2)^2)C_2/(C_1+C_2) \quad 6.4$$

if $(C_1 + C_2) > 8$.

In order to simplify matters, assume that for all flowerheads in which super-oviposition has occurred, the proportion of larvae surviving up to third instar is 0.34, and that therefore equation 6.4 holds. In order to reduce the number of variables in the fitness function, and therefore to reduce computing time, assume also that the size of clutch already oviposited before super-oviposition is eight. The simplified super-oviposition survival equation then becomes:

$$W_2(C) = 0.34(-0.0396 + 1.02(8+C_2) - 0.0204(8+C_2)^2)C_2/(8+C_2) \quad 6.5$$

Equations 6.2 and 6.5 are equivalent to Equations 4.1 and 4.2 in Table 1. The solutions of these two equations for clutch sizes c = 0 to 8 form matrices which are included within the MAX{ } functions of the Dynamic Modelling Equation.

Encounter probabilities: l_0, l_1, l_2

Turning now to the probability functions which describe encounters and non-encounters with flower head hosts, ie the functions $l_0(t)$ and $l_1(t)$, $l_2(t)$, Mangel and Clark (1988 chapter 4) point out that there is a relationship between the number of clean hosts and parasitised hosts, so that:

$$N_2(t) = N_0 - N_1(t) \qquad 7.1$$

where $N_2(t)$ is the number of parasitised hosts at time t, $N_1(t)$ is the number of clean hosts at time t, and N_0 is the total number of hosts. As clean hosts are parasitised, $N_2(t)$ increases and $N_1(t)$ decreases accordingly. In a random search model, the probability of a forager not encountering a host can be expressed as the first term of a Poisson expansion (Begon and Mortimer 1986). If e represents a random search parameter, and N_0 the number of hosts, then the probability of not encountering a host in time interval t is (Mangel and Clark 1988, chapter 4):

$$l_0(t) = \exp(-eN_0) \qquad 7.2$$

The probability of encountering a host of type N_1 in time interval t can then be written in the following way (Mangel and Clark 1988 Chapter 4 4.17):

$$l_1(t) = (1 - \exp(-eN_0))N_1(t)/N_0 \qquad 7.3$$

The random search parameter, e, can be derived as follows. In Chapter 4 it was found that the rate of encountering flower buds is one encounter every 4.2 minutes. The shortest time between ovipositions was observed to be approximately 15 minutes, which is taken as the length of each time interval in the dynamic model. In this time, an average of approximately three or four flower buds could be visited as the fly searches through a patch. If the total number of flower buds, including those suitable for oviposition, those which are still enveloped by leaves and those which have passed the stage suitable for oviposition, in the patch is taken as S, then the probability of a given fly encountering a given suitable host will be 1/S. In four encounters say, assuming that the flower buds can be revisited, the probability of encountering a given suitable host is 4/S, which is equivalent to the random search parameter, e (Begon and Mortimer 1986: Section 5.13).

In order to specify the value of e for the purposes of the model, the total number of flower buds in a patch needs to be stated. The mean density of flowerheads over a period of four years was found to be approximately 120 flower buds per metre square (Chapter 10, Table 57). In Chapter 4 it was observed that flies may travel up to 1.6 metres between flower bud encounters. The area within which a fly may encounter any flowerhead, is thus described by a circle of radius 1.6 metres, which gives an area of approximately 8 square metres. At 120 flower buds per square metre, it would be reasonable to set the patch size at about 1000 flower buds, giving a random search parameter, e = 0.004.

7.1.2 Solving the Dynamic Modelling Equation

The purpose of this equation is to calculate the Maximum Lifetime Expected Fitness (F(n,x,t,T)), for every value of the variables in the equation. The fly dies in the final time interval, T, so the solution for all variables in the final time interval is defined as zero:

$$F(n,x,T,T) = 0$$

Table 29: Solutions of a simplified Dynamic Modelling Equation for the last four time intervals, and for egg loads of 0, 1 and 2 eggs. The fly may oviposit only one egg in each time interval. p(c,x,t) = 1; $l_0(t)$ = 0.5; $l_1(t)$ = $l_2(t)$ = 0.25; $W_1(1,x,t)$ = 0.75; $W_2(1,x,t)$ = 0.5. So for example, for t = T-3 and x = 2, substitute 0.62495 for F(x,t+1,T) and 0.4685 for F(x-1,t+1,T). The clutch size giving these solutions is also shown.

$$F(x,t,T) = 0.5.F(x,t+1,t) + MAX\{0.25(0.75 + F(x-1,t+1,T); F(x,t+1,T)\} + MAX\{0.25(0.5 + F(x-1,t+1,T); F(x,t+1,T)\}$$

Eggload	0	1		2	
Time interval	F(x,t,T)	F(x,t,T)	Clutch	F(x,t,T)	Clutch
T	0	0	0	0	0
T-1	0	0.3125	1	0.3125	1
T-2	0	0.4685	1	0.62495	1
T-3	0	0.54675	1	0.859725	1

This solution can then be fed into the equation, and the solutions for all values of the variables, for the preceding time interval, T-1, can be calculated. Similarly, the solution for time interval T-2 is calculated by including the solution for time interval T-1, in the equation. In this way, by working backwards in time from the final time interval, through each time interval to the initial time interval, solutions of the equation for all values of the variables in all time periods can be calculated (see Table 29). The result is a matrix of solutions for F(n,x,t,T) at all values of n, x, and t.

In the Dynamic Modelling Equation (Table 28, Equation 1), the MAX{ } functions calculate the maximum value of the nine terms in the brackets. The nine terms correspond to clutch sizes c = 0 to 8. Thus, if $MAX\{W_1(c,x,t) + F(n+1,x-c,t+1,T)\} = W_1(3,x,t) + F(n+1,x-3,t+1,T)$ then a clutch of size three will be oviposited in a non-parasitised flowerbud. If $MAX\{W_2(c,x,t) + F(n,x-c,t+1,T)\} = W_2(0,x,t) + F(n,x,t+1,T)$, then super-oviposition will have been avoided in a parasitised flowerbud. It is possible therefore to use the MAX{ } functions in order to set up a matrix of optimal oviposition decisions, corresponding to the matrix of Maximum Expected Lifetime Fitness (see Table 29).

Development of the computer programme

The computer programme is written in Locomotive Basic 2, on a 640K RAM personal computer. The programme with explanations is given in the Appendix.

Structure of the programme

1 Arrays are defined and the initial number of hosts is set.

2 The Final Time Interval lifetime expected fitness is defined as zero for all eggloads. Lifetime Expected Fitness is calculated iteratively from the Final Time Interval back to the Initial Time Interval, for all eggloads and for the whole range of host distributions. This process produces the expected fitness array, which gives information about optimal individual decisions concerning egg load and super-oviposition.

3 A "Monte Carlo" simulation cycles through all time intervals from the Initial to Final Time Intervals, for a range of fly numbers, in order to look at population level phenomena.

i A random number (z) is assigned to one of three encounter possibilities: no encounter, encounter with non-parasitised host, encounter with parasitised host. If $0 <= z <= l_1(t)$ then there is an encounter with a non-parasitised host; if $l_1(t) < z <= l_1(t) + l_2(t)$ then there is an encounter with a parasitised host; if $l_1(t) + l_2(t) < z <= 1$ then there is no encounter.

ii If a parasitised host is encountered a second random number determines the reference number of the host which is encountered, so that the number of eggs laid in the host can be computed.

iii The oviposition and clutch size decision is ascertained for the type of host encountered, by reference back to the Expected Lifetime fitness array, and is printed out.

iv The egg load of each insect, the number of non-parasitised and parasitised hosts are calculated at the end of each time interval, and carried over into the next time interval. If a new host is parasitised it is assigned a reference number and the clutch size oviposited in it is logged. If a parasitised host is encountered, the total number of eggs oviposited into it is logged.

4 At the end of the Monte Carlo simulation, the egg load of each insect, the number of eggs laid in each host, the mean and variance of number of eggs in hosts, and proportion of hosts parasitised are printed out. This output gives a summary of the population level consequences of the summed optimal behaviour of the flies in the simulation.

The presentation of results of the model is in two parts. In the next section of this chapter, the results of running the first part of the programme, which computes the array of Lifetime Expected Fitness, are discussed. Predictions concerning the optimal individual behaviour of female gall flies, as determined by the Lifetime Expected Fitness array, are considered. One prediction of the Dynamic Modelling Equation is then tested experimentally in the final section. In Chapter 8, results of the Monte Carlo Simulation, which refer to population level phenomena, are compared with some field data. The model is then used to explore what might happen if field densities of gall flies are increased.

Section 2: Predictions of the model concerning optimal behaviour of individuals

7.2.1 Introduction

In this section some of the predictions of the Dynamic Modelling Equation developed in the previous section concerning the individual behaviour of ovipositing female gall flies are presented, and compared to the predictions of similar dynamic models (Mangel and Clark 1988, Chapter 4).

The model combines the elements of two fundamental decisions an ovipositing female gall fly needs to make:

1 Whether to accept or to reject parasitized hosts.

2 How many eggs to oviposit.

Two optimal decisions of individual flies are investigated here in relation to the model: 1) whether to oviposit clutches of less than eight eggs, which is the single clutch maximum; and 2) whether to reject hosts which are already parasitized. The model is first explored using the fitness and search parameters, derived from field data in Chapter 2, at varying host densities.

Gladstein et al (1991) argue that because Dynamic Models do not have a general solution, it is difficult to assess the validity of their predictions unless a sensitivity analysis is also carried out. Sensity analysis can be of two types (Gladstein et al 1991): 1) running the model at different

parameter values, in order to identify trends in response to changing parameters, and 2) comparing the outcome of the model when organisms consistently choose a sub-optimal strategy. In order to test the present model's sensitivity to changing parameters, the first part of the programme was run at all the combinations of five encounter rates (e), and five values of the constant of the quadratic term of the fitness functions $W_1(c,x,t)$ and $W_2(c,x,t)$ (called the "fitness curvature"). The host density was held constant, and the output was considered for the 40th time interval before the Final Time Interval. Sensitivity to a sub-optimal strategy is considered in Chapter 10.

7.2.2 Method

The first part of the computer programme, as shown in Tables 1 and 2 of Appendix 2, was run. This part of the programme computes the array of solutions of the Dynamic Modelling Equation. In addition, lines were added to the programme so that the Optimal Clutch Size corresponding to an encounter with a non-parasitized host, and also that corresponding to an encounter with a parasitized host were recorded. A Clutch Size of zero is interpreted as refusal to oviposit.

The programme was first run at 15 different initial host distributions. The optimal clutch size was printed out for four values of eggload (10, 25, 45, 64) at each of three time intervals before the Final Time Interval (10, 40, 70) for each of the 15 initial host distributions (Tables 30 and 31). The objective was to explore how clutch size and rejection or acceptance of parasitized hosts vary with egg load, time interval, and host distribution.

The next set of trials kept the host distribution at 50 non-parasitized and 10 parasitized hosts, and recorded the egg load at which the fly began to oviposit clutches of eight eggs in non-parasitized hosts, and rejected parasitized hosts, for every fifth time interval working back from the Final Interval. The objective was to obtain isoclines on the time interval/eggload plane for rejection/acceptance of parasitized hosts, and for maximum clutch size in non-parasitized hosts, at constant host distribution.

The third set of trials varied the number of non-parasitized hosts between 5 and 80 while keeping the number of parasitized hosts constant at 10. The egg load at which a fly began to oviposit clutches of eight eggs in non-parasitized hosts, and rejected parasitized hosts, in the fortieth time interval working back from the Final Interval was recorded. The objective was to obtain similar isoclines on the non-parasitized host number/egg-load plane, at constant time interval before the Final Interval.

The fourth set of trials ran the model at the 25 combinations of five encounter rates and five values of the "fitness curvature", at 50 non-parasitized hosts and 10 parasitized hosts. The output at the 40th time interval before the Final Time Interval indicated the eggload at which the fly would begin to oviposit clutches of less than eight, and would reject parasitized hosts.

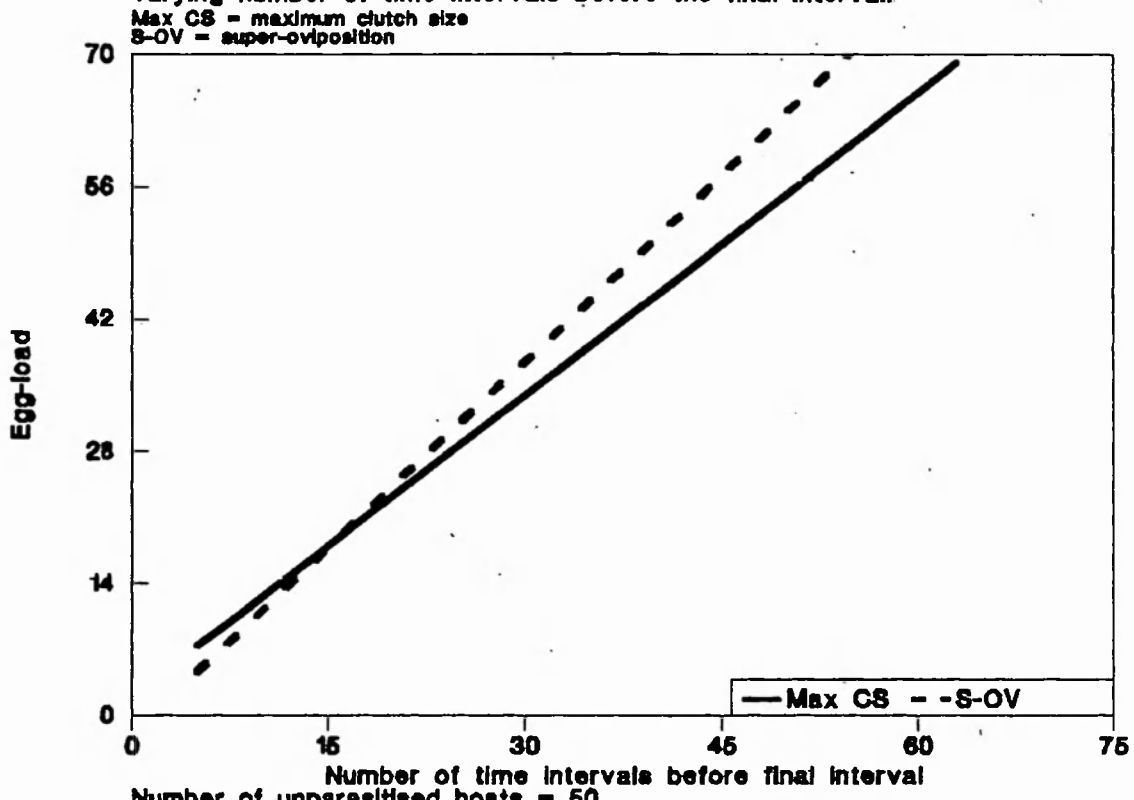
7.2.3 Results

The results of the first set of trials (Tables 30 and 31), confirm that the model predicts varying individual responses to encounters with the two types of host at different numbers of time intervals before the Final Time Interval, eggload and host distsibution. Zero clutches (rejection of a host) occur only with parasitized hosts. Non-parasitized hosts are always accepted if encountered. Super-oviposition does occur under certain conditions. Clutch sizes in both non-parasitized and parasitized hosts become smaller as the number of time intervals before the final interval increases, as the egg load decreases, and as the number of hosts increases.

The isoclines of parasitized host rejection/acceptance and of ovipositing clutch size 8, obtained from the second set of trials (Figure 8) approximate well to a linear relationship between number of time intervals before the final interval and egg load. At high egg-load and small number of time intervals before the final interval, superovipostion and clutch sizes of eight in non-parasitized hosts are predicted. In a narrow range of egg-loads for each time interval greater than 16, clutch sizes of eight will be oviposited in non-parasitized hosts, and parasitized hosts will be rejected.

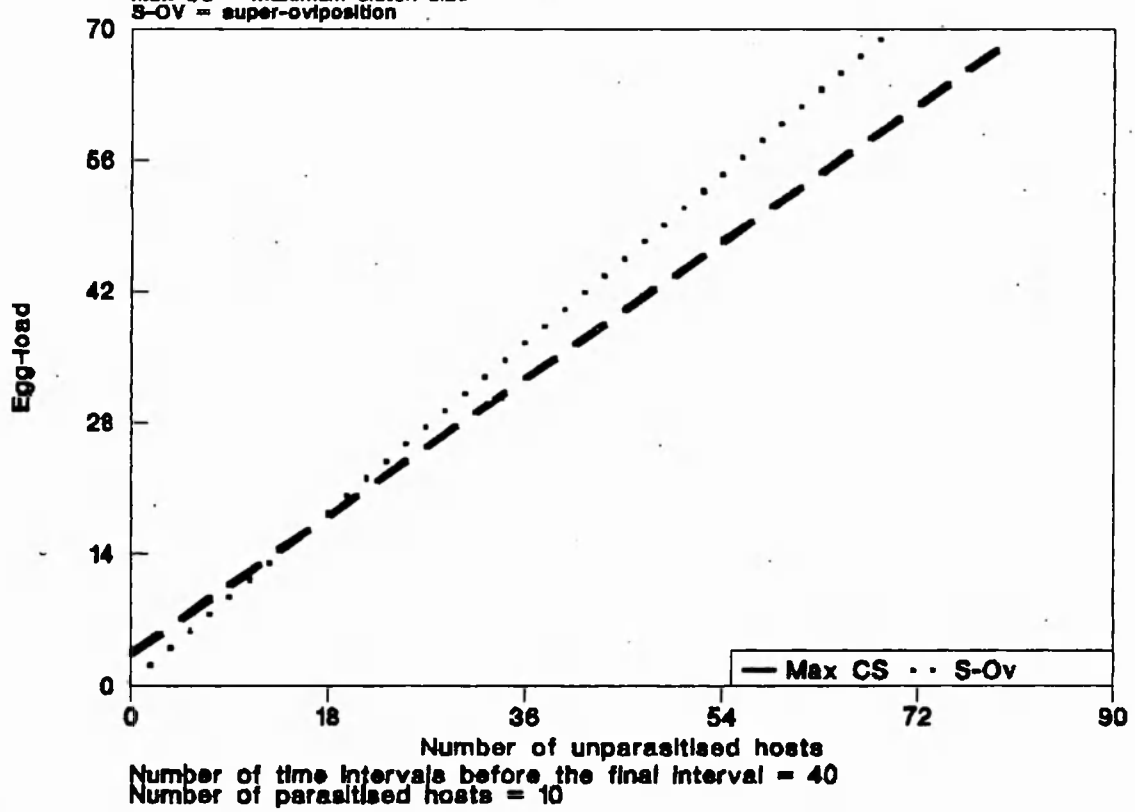
The isoclines obtained from the third set of trials (Figure 9) approximate to a linear relationship between number of non-parasitized hosts and egg-load. At low numbers of non-parasitized hosts, super-oviposition and maximum clutches will occur at lower egg-loads, than at high host numbers.

Figure 8: Egg-load above which 1) the maximum clutch size is oviposited in unparasitised hosts, and 2) super-oviposition occurs in parasitised hosts, at varying number of time intervals before the final interval.



Number of unparasitised hosts = 50
Number of parasitised hosts = 10

Figure 9: Egg-load above which 1) the maximum clutch size is oviposited in unparasitised hosts, and 2) super-oviposition occurs in parasitised hosts at varying host numbers.



Number of time intervals before the final interval = 40
Number of parasitised hosts = 10

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Table 30: Results of trials of dynamic model:

Optimal clutch sizes predicted to be oviposited in non-parasitized hosts

The dynamic modelling programme was run at 15 host distributions, and the optimal clutch size computed at time intervals 10, 40, and 70 before the final interval, at eggloads 10, 25, 45, and 64.

NPar = number of non-parasitized hosts; Par = number of parasitized hosts

Time Interval		10				40				70			
Eggload		10	25	45	64	10	25	45	64	10	25	45	64
Host dis.		Optimal clutch size:											
NPar	Par												
10	10	8	8	8	8	7	8	8	8	5	8	8	8
25	10	8	8	8	8	4	8	8	8	2	5	8	8
50	10	6	8	8	8	2	5	8	8	2	3	4	6
100	10	4	8	8	8	2	2	8	6	1	2	2	3
150	10	3	6	8	8	1	2	3	4	1	1	2	2
200	10	2	5	8	8	1	2	2	3	1	1	2	2
250	10	2	4	7	8	1	1	2	3	1	1	1	2
10	25	8	8	8	8	6	8	8	8	5	8	8	8
25	25	8	8	8	8	4	8	8	8	2	5	8	8
50	25	6	8	8	8	2	5	8	8	2	3	4	6
100	25	4	8	8	8	2	2	4	6	1	2	2	3

100	100	4	8	8	8	2	3	5	6	1	2	3	4
150	150	3	7	8	8	1	2	4	5	1	2	2	3
200	200	3	6	8	8	1	2	3	4	1	1	2	3
250	250	3	6	8	8	1	2	3	4	1	1	2	2

Table 31: Results of trials of dynamic model:
Optimal clutch sizes predicted to be oviposited in parasitized hosts

The dynamic modelling programme was run at 15 host distributions, and the optimal clutch size computed at time intervals 10, 40, and 70 before the final interval, at egg loads 10, 25, 45, and 64.

NPar = number of non-parasitized hosts; Par = number of parasitized hosts

Optimal clutch size 0 means super-oviposition is rejected.

Time Interval		10				40				70			
Eggload		10	25	45	64	10	25	45	64	10	25	45	64
Host dis.		Optimal clutch size											
NPar	Par												
10	10	7	8	8	8	1	7	8	8	0	2	8	8
25	10	3	8	8	8	0	1	8	8	0	0	0	5
50	10	1	8	8	8	0	0	0	5	0	0	0	0
100	10	0	1	8	8	0	0	0	0	0	0	0	0
150	10	0	0	6	8	0	0	0	0	0	0	0	0
200	10	0	0	3	8	0	0	0	0	0	0	0	0
250	10	0	0	0	8	0	0	0	0	0	0	0	0

10	25	5	8	8	8	0	4	8	8	0	1	4	8
25	25	3	8	8	8	0	0	5	8	0	0	0	3
50	25	0	7	8	8	0	0	0	3	0	0	0	0
100	25	0	1	8	8	0	0	0	0	0	0	0	0
100	100	0	0	6	8	0	0	0	0	0	0	0	0
150	150	0	0	3	8	0	0	0	0	0	0	0	0
200	200	0	0	2	6	0	0	0	0	0	0	0	0
250	250	0	0	1	5	0	0	0	0	0	0	0	0

Results of sensitivity analysis

The sensivity trials show that as the "fitness curvature" increases at a given encounter rate, the clutch size oviposited in unparasitised hosts noticeably decreases (Figure 10). The egg load at which parasitized hosts are rejected only slightly decreases (Figure 11) for each of the five encounter rates. Increasing "fitness curvature" has the effect of increasing the increments by which per capita survival up to gall formation decrease with clutch size. The overall effect of increasing "fitness curvature" is that flies will tend to oviposit smaller clutches at a given egg load. Rejection/acceptance of parasitized hosts is less sensitive to varying this parameter because its main determinent is the per capita survival post-gall formation.

Increasing the encounter rate, while holding the "fitness curvature" constant, only slightly decreases the clutch size at a given egg load (Figure 10). The egg load at which parasitised hosts are rejected

increases significantly with search efficiency (Figure 11). These results are as expected, because increasing encounter rate has the same effect as increasing host density.

7.2.4 Predictions of the Dynamic Modelling Equation about individual behaviour

1 **Varying Time Interval before End Time**

1.1 As the number of time intervals before the Final Time Interval becomes less, so, according to Table 30, the size of clutch oviposited in non-parasitized hosts becomes greater, up to a maximum of eight eggs.

1.2 As the number of time intervals before the Final Time Interval becomes less, it is more likely that superoviposition will occur.

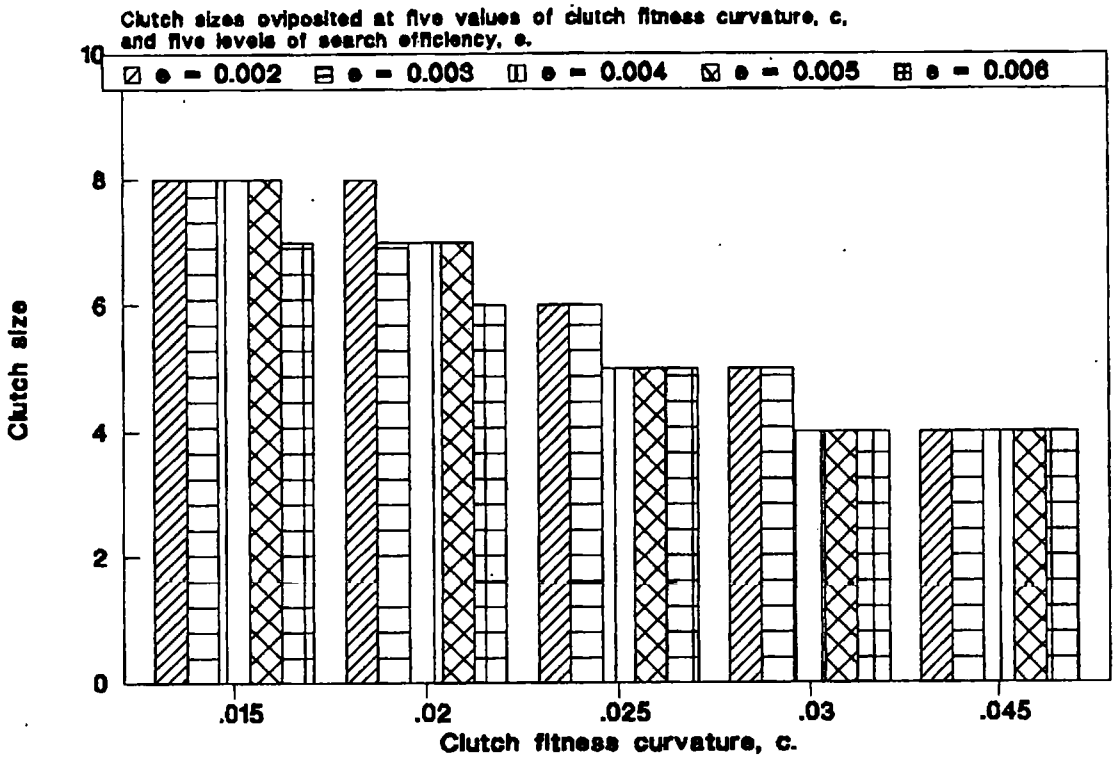
1.3 If superoviposition occurs then the second clutch becomes larger the nearer is the Final Time Interval.

2 **Varying Eggload**

2.1 The larger the eggload, the larger is the size of clutch oviposited in clean hosts, at constant host distribution and number of intervals before the Final Time Interval.

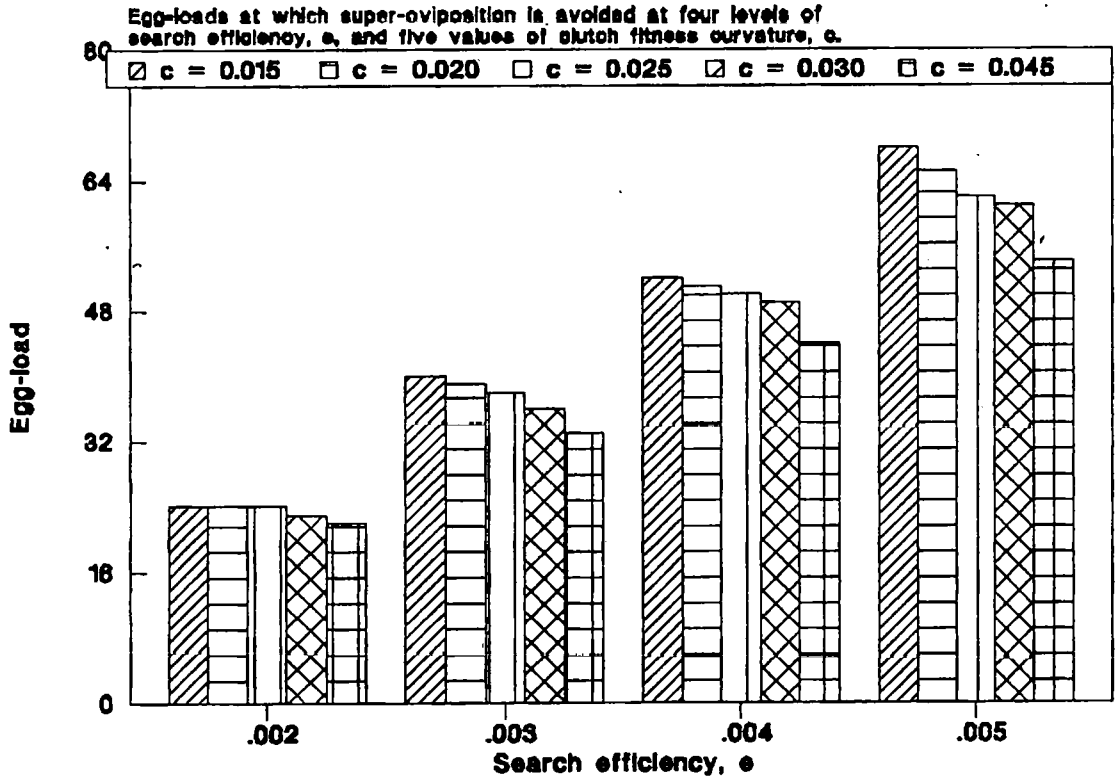
2.2 The larger the eggload, the more likely it is that superoviposition will occur in parasitized hosts.

Figure 10: Sensitivity Analysis I.



Egg-load = 32
Number of time intervals before final time interval = 40
Number of hosts: unparasitized = 60; parasitized = 10

Figure 11: Sensitivity Analysis II.



Number of time intervals before final interval = 40
Number of hosts: unparasitised = 60; parasitised = 10

2.3 If superoviposition occurs, then the larger the eggload, the larger is the size of the second clutch.

3 **Varying the distribution of hosts**

3.1 Superoviposition is more likely to occur at low numbers of clean hosts, and to be avoided at higher numbers of clean hosts.

3.2 At high numbers of clean hosts, the size of clutches oviposited in clean hosts is lower than at low numbers of clean hosts.

7.2.5 Discussion

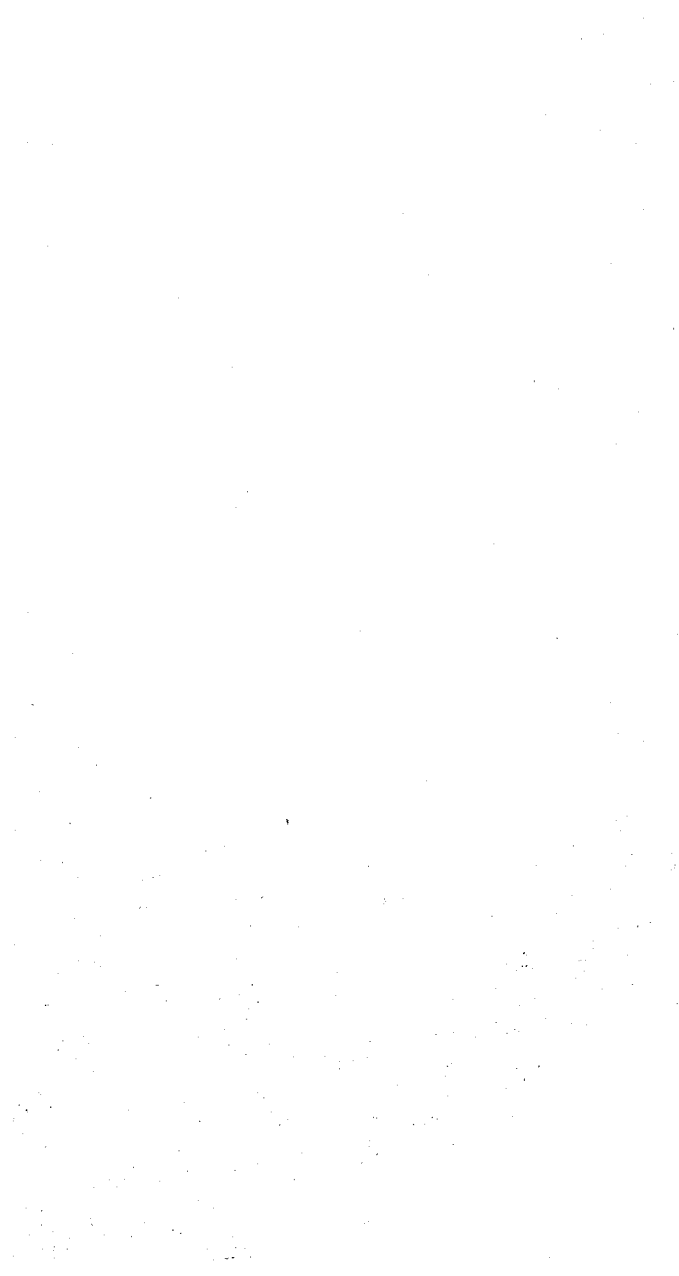
In general terms these predictions of the Dynamic Modelling Equation concur with the predictions of the two models presented by Mangel and Clark (1988, Chapter 4) and with that of Clark (1987). The set of predictions are also consistant with the models of Weisser and Houston (1993) and Collier (1995). In this respect, the model presented here adds nothing new to the predictions concerning the individual behaviour of flies. As expected, clutch size and host acceptance depend upon the egg load and age, and the number of hosts available for oviposition.

The model uses parameters which have been derived in previous chapters concerning per capita survival of eggs and larvae in relation to clutch size, and also encounter rates with hosts in the field. Varying these parameters, while affecting the precise optimal behavioural decision for a given egg load, time interval and host density, do not affect the qualitative predictions set out above.

Other parameters are less empirically based, such as initial egg load, foraging lifespan of flies, and probability of survival. The model also relies on a fundamental assumption about the physiology of the insect: that it is pro-ovigenic. Mangel (1987) suggests that such an assumption is not too unrealistic for some diptera, although further investigation of the ovarian dynamics of *Urophora jaceana* would be desirable.

These estimates and assumptions are obviously a weakness in the present model, although adding more state variables into the model, such as a probability of survival function, would inevitably increase complexity and make the computing time required extremely long.

While conscious of these limitations, the basic dynamic modelling equation provides the basis of a workable model. In the next section, the prediction that super-oviposition is more likely to occur at low numbers of clean hosts, and to be avoided at higher numbers of clean hosts is tested experimentally. In Chapter 8 the Monte Carlo part of the model is used to simulate the consequences of fly behaviour at the population level, which are then compared with field data.



Section 3: Super-oviposition in relation to the frequency of encountering a host

In this section, an important prediction of dynamic models which include hosts of varying qualities is tested. In this case the prediction refers to the likelihood of gall flies ovipositing in hosts in which oviposition has already occurred, (super-oviposition), at different rates of encounter with suitable hosts. The aim is to provide an independent experiment against which predictions of the dynamic model may be tested.

7.3.1 Introduction

Super-oviposition, ie oviposition in a host which has already been parasitised, has been observed in parasitoids and in tephritid flies (Mangel 1992). The strategy of marking hosts with oviposition deterring pheromones (Roitberg and Prokopy 1987) enables ovipositing females to discriminate more efficiently between hosts. Data from parasitoids, and herbivores, for example Rhagoletis, orange tip butterfly, indicate that a parasitised host is of lower quality than an unparasitised host, giving a lower per capita survival of offspring. If the insect's strategy is to maximise fitness per clutch (Lack 1947), then it would avoid super-oviposition. However, it is now well established that maximising lifetime expected fitness should be the guiding principle in considering host acceptance (Mangel and Clark 1988). In attempting to maximise expected lifetime fitness, under certain conditions it would be advantageous for an insect to super-oviposit rather than to reject a parasitised host.

A series of experiments, mentioned in Chapter 4, on host acceptance by *Rhagoletis pomonella*, which marks fruit it has parasitised (Averill and Prokopy 1987), have investigated the threshold between accepting and rejecting marked hosts. Roitberg and Prokopy (1983) found that the tendency to oviposit in marked hosts increased with greater host deprivation. In a second experiment, Mangel and Roitberg (1989) included egg load as a variable in determining whether a fly would accept or reject a marked host, in addition to the time since last oviposition and the fraction of marked to unmarked hosts. They found that the inclusion of egg load as a variable does help to explain the occurrence of super oviposition. Flies with larger egg loads should have a greater tendency to super-oviposit than flies with smaller egg loads. This is because in order to maximise lifetime fitness, as many eggs as possible must be oviposited. With large numbers of eggs available, this means accepting some hosts of lesser quality.

This second experiment, and the theoretical framework in which it is analysed, points up the importance of including egg load as variable in host acceptance studies. Indeed, one of the main predictions of dynamic state variable models is that the oviposition decisions an insect makes is dependent on egg load (Mangel and Clark 1988, Mangel 1987, Iwasa et al 1984). In their review, Minkenberg, Tatar and Rosenheim (1992) identified a number of empirical studies in which egg load appeared to contribute to changes in insect foraging and oviposition behaviour. However, they drew attention to the particular problem of isolating egg load from other physiological variables of the insects. Most difficult is the separating out of influences due to egg load from those due to the experience of the fly, particularly in host deprivation experiments. In an experiment

which does successfully separate out these two variables (Rosenheim and Rosen 1991), both egg load and experience were found to influence oviposition decisions.

In the experiment described below one important prediction of dynamic state variable models, concerning the tendency of insects to super-oviposit, is tested against the behaviour of *Urophora jaceana* females ovipositing in flowerbuds of *Centaurea nigra*. The prediction is that:

> At low densities of suitable hosts for oviposition, a female gall fly will be more likely to super-oviposit in hosts in which eggs have already been laid, than at high densities of suitable hosts.

A practical method was needed to simulate high and low densities of hosts, and which would enable ovipositions to be observed and recorded. Roitberg and Prokopy (1984) presented individual experienced flies with different sequences of marked and unmarked hosts. Flies were presented with one host at a time. In this instance, the prediction that search persistence in a patch would be reduced after a fly had been presented with a sequence of marked hosts was being tested. This method of presenting one host at a time in a known sequence of encounters is adapted in the experiment described below.

7.3.2 Method

Sufficient numbers of flowerheads in which it was certain there had been no previous oviposition were made available from a stock of plants kept in the garden. It was hoped to be able to use virgin gall flies

emerged from galls kept in 2cm pots, but few gall flies emerged from the galls kept in these pots. It was necessary then to use wild gall flies from the field. Unfortunately, there was no way of determining the age or recent experience of the gall flies. In order to minimise the effect of recent experience of oviposition, gall flies captured from the field were kept for 24 hours before being used in the experiment. In this way, the recent experience of all gall flies was of non-oviposition and no encounters with hosts. They were kept in 2cm pots with one male at room temperature.

In each replication of the experiment, the gall fly was observed continuously while it was was allowed to encounter a predetermined sequence of hosts (Table 32). Flowerbuds suitable for oviposition were held on white card squares with a central hole through which the stalk of the bud was pushed. The card was placed with the bud upright over a 2cm plastic pot filled with tap water, so that the stalk of the bud reached the water. Above the card, there was approximately 2cm of stalk showing, with the bud enveloping leaves intact.

The gall fly was contained in a 2cm clear plastic pot inverted over the flower bud, resting on the card. Gall flies could be easily transferred from one bud to another in the sequence using a pooter fitted with a 2cm pot. Each fly was allowed 15 minutes in which to search, probe and decide whether or not to oviposit in the host provided. As reported elsewhere (Chapter 2.2), the minimum search period for gall flies appears to be 15 minutes.

Positive encounters only were allowed. An encounter was deemed positive, only if the gall fly alighted on the flower bud, walked over it, and probed with its oviscape. The encounter was considered to be finished when the gall fly left the flowerbud after probing or ovipositing. If a gall fly failed to make a positive encounter with a host within 15 minutes, it was removed from the experiment.

Oviposition, if it occurred, was allowed to continue uninterrupted, which resulted in the variable length of time each gall fly spent with a particular host.

After each encounter, the gall fly was transferred to the next part of the sequence. Sequence 1 (Table 32), representing a high frequency of encounter with clean hosts, consisted of encountering four flowerbuds in which no oviposition had occurred in succession, and the fifth encounter being with a parasitised host, a flowerbud in which oviposition had already occurred.

Sequence 2 (Table 32), representing a low frequency of encounter, consisted of first encountering a clean host, and then not encountering a host at all in the next three fifteen minute intervals. The final encounter was with a parasitised host.

Table 32 also includes the outcome, as predicted by the dynamic model, for each encounter. Oviposition should occur in all encounters with clean hosts. No oviposition can occur in an encounter with no host. Oviposition should be less likely to occur in an encounter with a parasitised host in high frequency sequence 1, than in low frequency sequence 2.

Table 32: Two sequences of host encounter with predicted outcomes

Sequence 1: High frequency		Sequence 2: Low frequency	
Encounter	Prediction	Encounter	Prediction
Clean	oviposition	Clean	oviposition
Clean	oviposition	None	no oviposition
Clean	oviposition	None	no oviposition
Clean	oviposition	None	no oviposition
Parasitised	no oviposition	Parasitised	super-oviposition

Note: clean = encounter with flowerbud in which oviposition has not previously occurred; parasitised = encounter with flowerbud in which oviposition has previously occurred; none = no host encountered in a fifteen minute interval.

After each replication of the experiment, all hosts and gall flies were preserved by freezing. Hosts were dissected under a binocular microscope, X 20, at a convenient time, and the number of eggs oviposited in each host was recorded. If two clutches could be identified, they were recorded separately. Gall flies used in the replications were also dissected, and the number of eggs present in the abdomen were counted and recorded. This was assisted by dispersing the eggs in a drop of tap water before counting them.

Dissection of hosts enabled confirmation that oviposition had or had not taken place, and dissection of gall flies enabled egg loads to be established.

7.3.3 Results

A total of 29 gall flies were used in the experiment. Of these, 7 were removed from the experiment because they failed to make an encounter with a host provided. This gives a total of 22 results, 11 with sequence 1 and 11 with sequence 2.

All 22 gall flies were observed to encounter hosts, and appeared to oviposit in all clean hosts which they encountered. The time taken to oviposit in a host varied substantially (Table 33), but all ovipositions began within 15 minutes of the beginning of the encounter period. In the high frequency sequence, super-oviposition was observed twice out of the eleven trials, and in the low frequency sequence, super-oviposition was observed ten times out of the eleven trials.

In the high frequency sequence, the number of eggs laid in clean hosts varied between one and eleven (Table 34). Although it appeared that oviposition occurred in all encounters with clean hosts, on dissection, eggs were not discovered in a number of such hosts. An asterisk in Table 3 indicates those hosts in which this was the case. In those cases for which no eggs were discovered in hosts, the flies did not have lower egg loads than average, possibly indicating that eggs were present but were not discovered. Alternatively, oviposition did not actually take place in these cases. The consequences of the latter for the experiment are discussed below.

Table 33: Super-oviposition experiment: The time of each encounter period for each replication of the experiment.

High frequency sequence. Time of beginning of encounter period (minutes):						Low frequency sequence. Time of beginning of encounter period (minutes):					
	1	2	3	4	5		1	2	3	4	5
Fly:						Fly:					
1	0	25	45	60	75	1	0	20	35	50	65
2	0	15	60	100	120	2	0	25	40	55	70
3	0	*	*	*	*	3	0	35	50	65	80
4	0	*	*	*	*	4	0	10	25	40	55
5	0	45	60	71	90	5	0	42	57	72	87
6	0	25	50	95	110	6	0	20	35	50	65
7	0	20	35	70	100	7	0	25	40	55	70
8	0	15	30	50	75	8	0	50	65	80	95
9	0	25	40	50	57	9	0	45	60	75	90
10	0	20	55	65	90	10	0	40	55	70	85
11	0	15	40	55	80	11	0	16	31	46	61

* represents replications for which times were not recorded.

The parasitised hosts used in the experiment were hosts in which other flies had previously oviposited. The number of eggs found in these hosts on dissection therefore contained a possible two clutches of eggs.

In the high frequency sequence, two flies were observed to oviposit in the parasitised hosts, and two clutches were discovered in each of these hosts. The number of eggs laid in the second ovipositions were three and one for flies nine and ten respectively. The first ovipositions in these hosts were seven and four, and were performed by other flies.

In the results for the low frequency sequence, ten flies were observed to super-oviposit and one not. In all but two cases two clutches were discovered on dissection of the hosts, and the smaller clutch was attributed to the second oviposition. In the two cases in which it was not possible to identify clearly two clutches, the number of eggs in the second clutch was taken to be half of the total number of eggs found.

In the high frequency sequence, the eggloads at the end of the sequence of encounters, shown in Table 34, display a wide variation, with a maximum of 105 eggs and a minimum of 22 eggs. The flies with the highest and the lowest egg load both avoided super-oviposition in the parasitised host. The two flies in this sequence which did super-oviposit, had similar egg loads (52 and 56). In the low frequency sequence, eggloads ranged between 16 and 95, with the one fly which avoided super-oviposition having an eggload of 82. The two flies with the lowest eggloads, of 16 and 21, both super-oviposited. it appears that there is no discernible pattern of super oviposition which is related to egg load. It is possible that egg load in this experiment is not an important factor. An analysis of variance was performed on the square root of the eggloads of the flies in the two sequences, producing an F value of 0.122, which is much less than the critical value, 4.38, at $p < 0.05$ with 19 degrees of freedom (Table 35).

Table 34: Results of super-oviposition experiment, indicating whether or not super-oviposition was observed in the final encounter of the sequence for each replication, and also the number of eggs oviposited in each oviposition.

High frequency sequence:

	Number of eggs oviposited in clean hosts.				No. eggs prior to experiment	Super-oviposition observed		Eggload at end
Host	1	2	3	4	in host 5	yes/no	No. eggs	
Fly:								
1	3	5	6	5	5	no	0	105
2	3	8	6	7	4	no	0	56
3	2	2	1	*	4	no	0	56
4	6	4	4	*	7	no	0	86
5	7	4	4	*	7	no	0	40
6	4	6	10	3	14	no	0	*
7	2	5	*	3	3	no	0	77
8	4	1	7	*	4	no	0	48
9	4	4	*	6	7	yes	3	52
10	5	5	6	8	4	yes	1	56
11	4	11	3	7	5	no	0	22

* represents flowerheads for which there are no results for number of eggs oviposited, but for which an apparent oviposition was observed.

Low frequency sequence:

	Number of eggs oviposited in clean hosts.	No. eggs prior to experiment	Super-oviposition observed		Eggload at end
Host	1	in host 2	yes/no	No. eggs	
Fly:					
1	7	1	yes	1	79
2	*	2	yes	2	92
3	5	4	yes	4	40
4	5	4	no	0	82
5	11	2	yes	1	95
6	6	5	yes	3	70
7	2	4	yes	3	16
8	2	3	yes	3	36
9	3	5	yes	3	21
10	5	2	yes	1	46
11	4	4	yes	4	51

* represents flowerheads for which there are no results for number of eggs oviposited, but for which an apparent oviposition was observed.

Table 35: Analysis of Variance Table comparing egg loads of flies in high and low frequency encounter sequences. Egg loads were transformed to square root. Normal error structure and identity link were used in the GLIM programme.

Source of deviance:	Sum of squares	Degrees of freedom	Mean sum of squares	Fs
Among treatments	0.39	1	0.39	0.12 ns
Within treatments	61.12	19	3.22	
Total	61.50	20		

	High frequency flies	Low frequency flies
Mean	59.80	57.09
Standard deviation	23.78	27.95

The number of super-ovipositions observed in flies of the high frequency sequence appears to be significantly less than in flies of the low frequency sequence. The GLIM programme was used, in order to test whether there is no difference in frequency of super-ovipositions between the two sequences. The value of G which the test gave was 11.97 (Table 36), which is significant at $p < 0.001$. The null hypothesis that there is no difference between the number of super-ovipositions must be rejected.

Table 36: Contingency table for super-ovipositions in sixth encounter. Expected values calculated by GLIM in brackets.

Sequence of encounters	Super-oviposition		No super-oviposition	
Sequence 1	2	(5.71)	8	(4.29)
Sequence 2	10	(6.29)	1	(4.71)

Scaled deviance = 11.97 df = 1 Significant at $p < 0.001$

Table 37: Contingency table for super-ovipositions in sixth encounter. Trials in which no eggs were discovered in the fourth encounter of sequence 1 have been omitted. Expected values calculated by GLIM in brackets.

Sequence of encounters	Super-oviposition		No super-oviposition	
Sequence 1	2	(4.26)	4	(1.77)
Sequence 2	10	(7.77)	1	(3.24)

Scaled deviance = 6.26 df = 1 Significant at $p < 0.025$

When those trials in the high frequency sequence in which no eggs were discovered in the fourth host presented to the fly are omitted, in order to remove any bias which may be due to non-ovipositing flies being presented with the fifth host, the value for G, as calculated by GLIM, is 6.26, which is significant at 0.025 (Table 37). The null hypothesis must therefore still be rejected.

7.3.4 Discussion

From the results of the experiment as they stand, the significant difference between the outcomes of the fifth encounter period of the high and low frequency sequences may be attributed to the sequence of encounters. When encounters with clean hosts are frequent, super-oviposition is less likely to occur, and when encounters with clean hosts are rare, super-oviposition is more likely to occur. This interpretation supports the prediction of the dynamic model, that at low densities of suitable hosts for oviposition, a female gall fly will be more likely to super-oviposit in hosts in which eggs have already been laid, than at high densities of suitable hosts.

Using gall flies captured in the field for this experiment had the disadvantage that age and experience of gall flies were indeterminate. It is possible that the gall flies with the very low egg loads at the end of the experiment were also the oldest and had experience of oviposition in the field, whereas those with the highest eggloads may have been correspondingly less experienced and younger. It is possible that these factors made the results of the experiment less reliable, although, as noted above, the eggload does not appear to affect the outcome of the trials.

The previous experience of the gall flies used in the experiment was also indeterminate, and may also affect the results. By keeping insects in pots for 24 hours before replications, it was hoped that previous experience would exert less of an influence. Within the experiment itself experience obviously differs between the two sequences. It is really the effect on oviposition strategy, of this difference in experience of encounters with hosts that the experiment was intended to test.

In four replications of the high frequency sequence, flies 3,4,5 and 8, no eggs were discovered on dissection of the fourth clean host in the sequence. As pointed out above, this may either mean that eggs were present but not seen, or that eggs were not present. Oviposition was considered to be observed in these cases. In each case, the insect remained in position for sufficient time to lay eggs. It is quite possible that in dissecting the flowerbuds, eggs were displaced and not discovered. But in any case, when these trials were omitted from the significance test (Table 37) a significant difference between the outcomes of the final encounter in the two sequences was detected.

However, the latter case may have implications for the validity of the results of the experiment, because if no oviposition actually took place in the fourth encounter with a host, then the gall fly may avoid oviposition in the fifth host whether it was parasitised or clean. It may be the case that gall flies will only decide to oviposit in hosts up to a limited number of encounters, even with clean hosts. The significantly fewer super-ovipositions performed by flies previously encountering four clean hosts may be attributed to this factor rather than to the flies' perception of host availability. Against this interpretation is the fact that in each encounter period, it was established that each fly was

actively searching for a host and probing. If the fly had reached a saturation limit, it would be more likely to rest and not to show any interest in the host. Ideally, this uncertainty about the correct interpretation of the results of the experiment should be investigated further, either by introducing a sixth encounter period in which a clean host is presented to the fly, or by introducing a third sequence of five clean hosts.

It would be possible to investigate other predictions of the model using these methods, by presenting gall flies with pairs of sequences which represent different extremes of conditions. It would be interesting to compare, for example, the outcome of a high proportion of parasitised hosts in a sequence compared to the outcome of a sequence of clean hosts. The model would predict that in this case super-oviposition is more likely to occur when there is a high proportion of parasitised hosts.

Chapter 8: The population Consequences of Individual Behaviour of Gall Flies.

In this chapter results of running the Monte Carlo Simulation part of the Dynamic Modelling Computer Programme (Appendix 3) are presented, with the aim of relating population level phenomena to the predictions of the Dynamic Modelling equation for individual behaviour.

8.1 Introduction

Monte Carlo Simulation enables individual behaviour to be translated into population effects, and has been used in a number of studies, including Mangel and Clark (1988), Mangel (1987), Roitberg et al (1992).

The procedure for the Monte Carlo Simulation is simple in principle, and follows the algorithm given by Mangel and Clark (1988, Chapter 4). The complete computer programme for the Monte Carlo Simulation is given in Tables 3, 4, 5 and 6 of Appendix 2. After the array of solutions of the Dynamic Modelling Equation has been calculated by backward iteration (see Chapter 7), the decisions of a cohort of "model flies" are followed through the time intervals by forward iteration, from the initial time to the final time interval. In each time interval, a random number is assigned to each fly, which determines whether an encounter with a host is made in that interval, and if so what type of host is encountered. The decision made by the fly in that encounter, will be the optimal one which results in the maximum lifetime expected fitness as defined in the array of solutions to the dynamic modelling equation.

At the end of each time interval, the state variables of the model are adjusted according to the decisions made by the cohort of flies. The egg load is calculated as the egg load of the fly at the beginning of the interval minus the clutch size oviposited during the interval. The density of unparasitised hosts is the density at the beginning of the interval minus the number of hosts parasitised in that interval by the whole cohort. In addition, parasitised hosts are each given a reference number so that if super-oviposition occurs, the total number of eggs oviposited in that particular host can be recorded.

At the end of each simulation, the percentage of hosts which are parasitised, and the mean and variance of the number of eggs oviposited in the hosts are printed out, representing population level data derived from the sum of individual decisions made by the cohort of model flies.

The usefulness of this procedure lies in the fact that both initial host density and the number of flies in the cohort can be varied for each trial, enabling a computer simulation experiment to be set up. Each trial, for a given initial host density and number of flies in the cohort, needs to be replicated (Mangel and Clark 1988, Chapter 4). In the first computer experiment, the density dependence of percentage parasitism and of mean number of eggs oviposited in hosts with respect to fly density and host density was investigated. Trials were replicated ten times.

The results gained from Monte Carlo Simulations, being numerical, do not have the generality of analytical models (Houston and McNamara 1992). Gladstein et al (1991) stress the importance of sensitivity analysis concerning the advantage to the organism of the optimal strategy over

alternative strategies. The number of alternative strategies is very great (Houston and McNamara 1992) and it is usually impractical to compare all possible strategies. In Chapter 7 sensitivity analysis was applied to the solution of the Dynamic Modelling Equation. In the second computer experiment in this chapter, the population effects of a cohort of insects following the sub-optimal strategy of always avoiding super-oviposition is considered.

There are other limitations of the Monte Carlo method, due to the fact that an approximation is built into it. In any one time interval in the model, individual flies behave independently of each other. Changes of host distribution due to oviposition decisions of other flies within the time interval are therefore not accounted for.

8.2 Method

In the first experiment the computer programme, including the Monte Carlo Simulation (Appendix) was run at initial host densities in steps of 25 between 25 and 300 hosts and at fly densities in steps of two between two and ten flies (Table 38). These densities are within the range observed in the field (Chapter 10). Each trial was replicated ten times, and the mean percentage of hosts parasitised, and the mean number of eggs per parasitised host calculated for each combination of fly and host density.

Sensitivity analysis (Gladstein 1991, Houston and McNamara 1992) was carried out by changing the computer programme so that the flies in the model population followed the sub-optimal strategy of always refusing to oviposit in parasitised hosts. The sub-optimal programme was

run at a density of six flies and at host densities of 25, 100, 200 and 300 hosts, with ten replications for each trial. The mean number of eggs per parasitised host arising out of the sub-optimal strategy for each combination of host and fly densities was then compared, using one way analysis of variance, to the mean number of eggs per parasitised host arising out of the optimal strategy for the same host and fly densities.

8.3 Results

The percentage of hosts parasitised was found to increase with fly density for a given host density (Table 38, Figure 12). The shape of the curve is convex, suggesting a density dependent relationship of percentage parasitism with fly density.

The mean number of eggs per parasitised host increases with fly density for a given host density (Table 38, Figure 13). The percentage of hosts parasitised gently decreases with increasing host number for a given fly density (Table 38, Figure 14).

The mean number of eggs per parasitised host decreases with increasing host density, at the three fly densities shown (Table 38, Figure 15). The decrease is rapid at lower densities, and flattens out at higher densities.

Figure 12: Results of Monte Carlo Simulation I.
The percentage of hosts parasitised with varying number of flies at four initial host numbers.

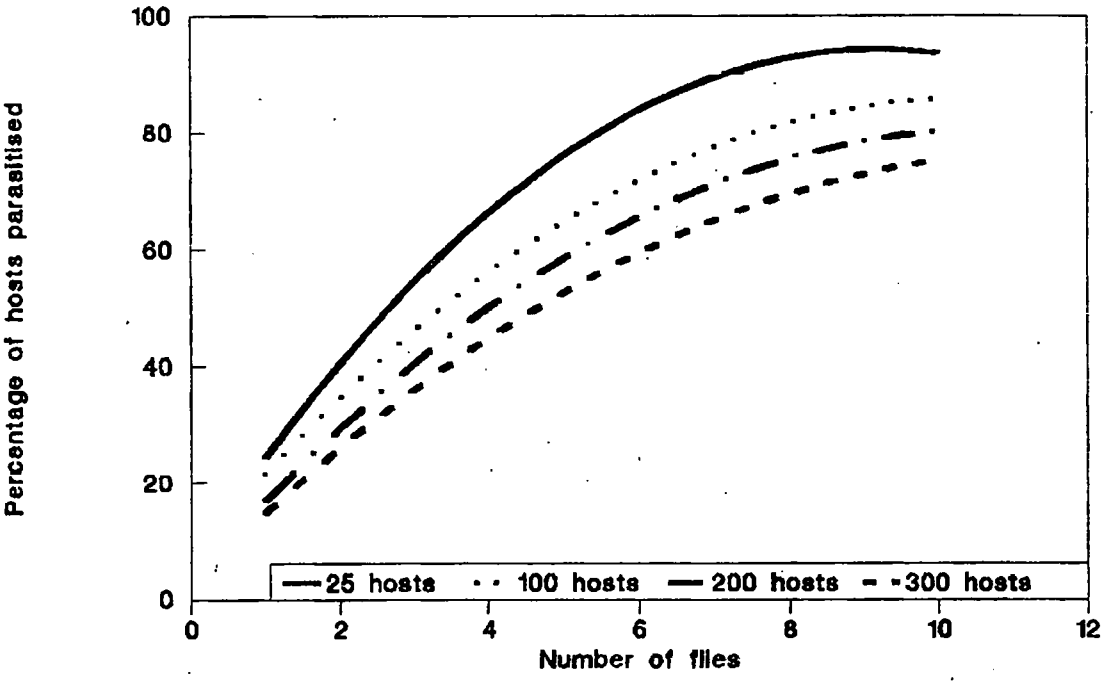
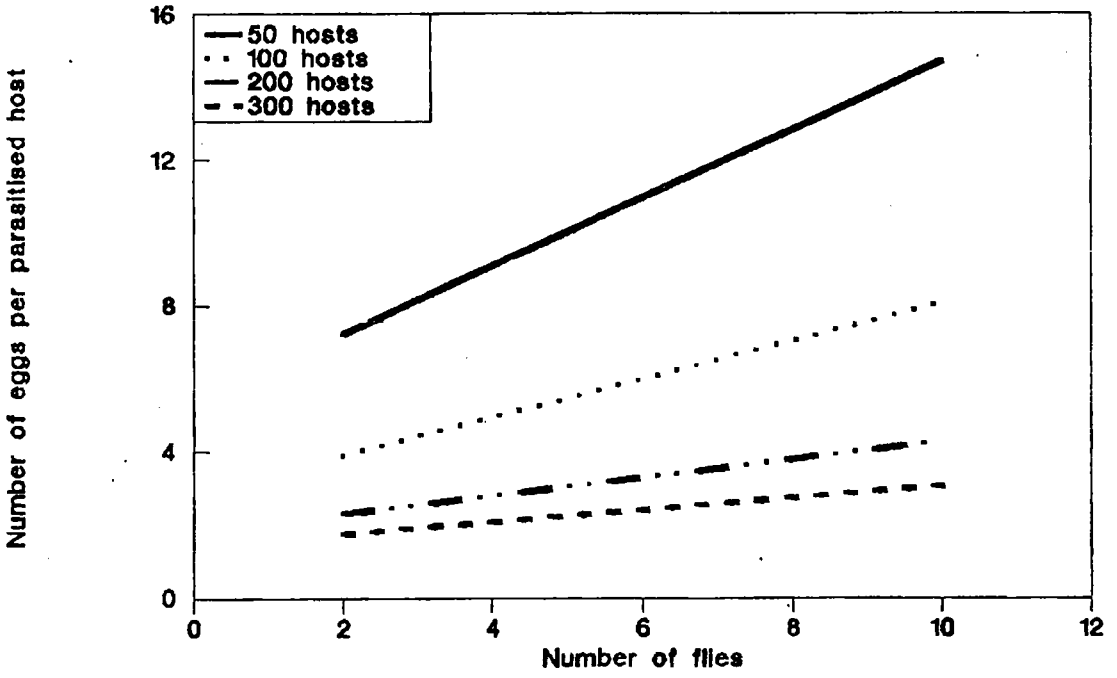
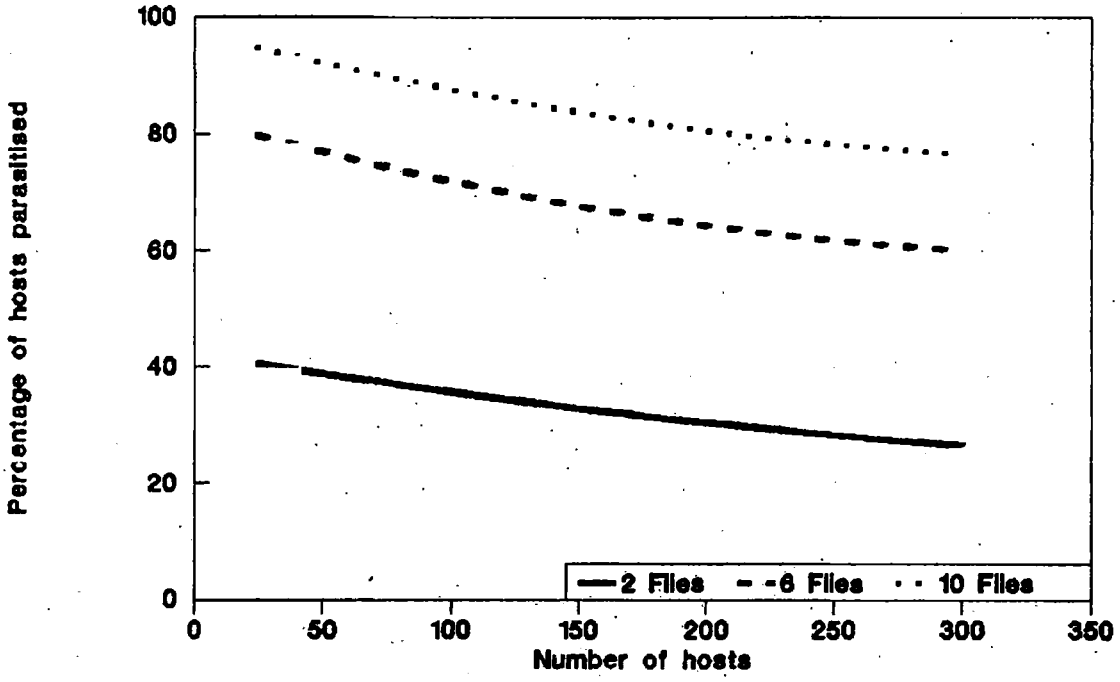


Figure 13: Results of Monte Carlo Simulation II.
Number of eggs per parasitised host at varying numbers of flies and at four host numbers.



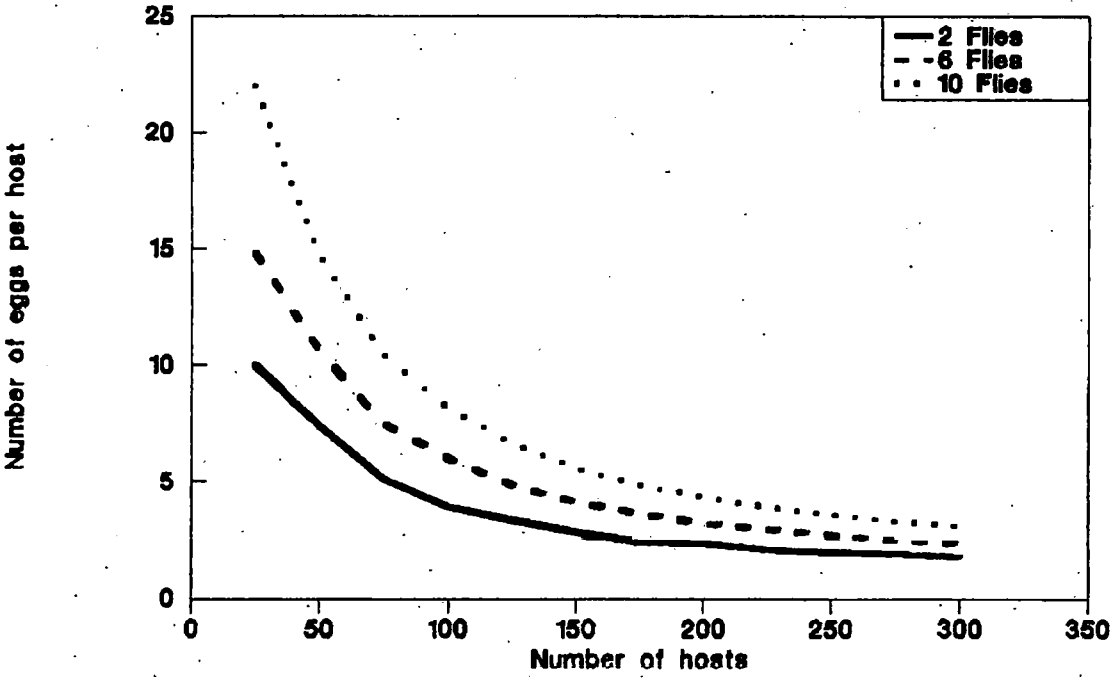
Data points are the mean of 10 trials

Figure 14: Results of Monte Carlo Simulation III.
The percentage of hosts parasitized against host number at three fly densities.



Each data point is the mean of ten trials of the Monte Carlo simulation.

Figure 15: Results of Monte Carlo Simulation IV.
The number of eggs per parasitized host against host number at three fly densities.



Each data point is the mean of ten trials.

Table 38: Mean and standard errors of 1) percentage of hosts parasitised and 2) number of eggs per parasitised host for each combination of fly and host densities used in the Monte Carlo Simulation Trials.

	2 Flies	4 Flies	6 Flies	8 Flies	10 Flies
25 Hosts	42.0 (1.3)	71.6 (2.0)	80.4 (2.6)	90.4 (1.7)	95.6 (1.2)
	10.0 (.38)	12.4 (.43)	14.8 (.44)	20.0 (.48)	22.0 (.66)
50 Hosts	37.6 (1.8)	60.4 (1.2)	77.0 (0.7)	84.4 (1.1)	92.6 (0.9)
	7.4 (.32)	9.0 (.14)	10.6 (.13)	13.1 (.16)	14.7 (.11)
75 Hosts	36.5 (0.9)	58.3 (0.7)	74.3 (0.7)	84.4 (1.1)	88.9 (0.9)
	5.1 (.13)	6.3 (.09)	7.5 (.06)	8.8 (.11)	10.4 (.07)
100 Hosts	35.9 (1.0)	57.1 (0.6)	70.1 (0.7)	82.0 (0.4)	85.9 (0.9)
	3.9 (.11)	4.9 (.05)	6.0 (.06)	6.9 (.09)	8.1 (.09)
125 Hosts	33.5 (0.8)	55.4 (0.4)	69.4 (0.3)	78.8 (0.7)	84.7 (0.6)
	3.4 (.07)	4.0 (.02)	4.8 (.01)	5.7 (.05)	6.6 (.05)
150 Hosts	33.3 (0.5)	53.2 (0.5)	67.9 (0.3)	78.7 (0.5)	84.2 (0.3)
	2.8 (.04)	3.5 (.03)	4.1 (.02)	4.8 (.03)	5.5 (.02)
175 Hosts	32.9 (0.4)	51.9 (0.5)	66.5 (0.5)	75.9 (0.3)	82.9 (0.3)
	2.4 (.03)	3.1 (.03)	3.6 (.03)	4.2 (.02)	4.8 (.02)

200 Hosts	29.8 (0.4)	51.3 (0.4)	65.2 (0.4)	74.3 (0.4)	81.0 (0.3)
	2.4 (.04)	2.7 (.02)	3.2 (.02)	3.8 (.02)	4.3 (.01)
225 Hosts	30.0 (0.3)	49.7 (0.7)	63.0 (0.6)	73.2 (0.2)	80.0 (0.4)
	2.1 (.02)	2.5 (.03)	3.0 (.03)	3.4 (.01)	3.9 (.02)
250 Hosts	28.5 (0.5)	48.9 (0.6)	61.6 (0.4)	71.3 (0.4)	78.2 (0.3)
	2.0 (.03)	2.3 (.03)	2.7 (.02)	3.1 (.02)	3.6 (.01)
275 Hosts	27.2 (0.3)	47.6 (0.3)	61.3 (0.4)	69.7 (0.3)	77.5 (0.3)
	1.9 (.02)	2.1 (.01)	2.5 (.02)	2.9 (.01)	3.3 (.01)
300 Hosts	26.3 (0.3)	45.2 (0.4)	59.4 (0.3)	68.6 (0.4)	75.8 (0.2)
	1.8 (.02)	2.1 (.02)	2.4 (.01)	2.7 (.01)	3.1 (.01)

Note: For each combination of host and fly density, the top figure is the mean percentage of parasitised hosts, and the bottom figure is the mean number of eggs per parasitised host. Standard errors are in brackets. Means and standard errors were calculated from 10 trials for each combination of densities.

Results of Sensitivity Analysis

At host densities of 25 and 100 there is a significant reduction in the mean number of eggs per parasitised host when flies refuse to oviposit in parasitised hosts (Table 39). However, at host densities of 200 and 300 there is no significant difference in the mean number of eggs per parasitised host between the optimal and sub-optimal strategy.

Table 39: The mean and variance of the mean number of eggs per parasitised host of the optimal strategy compared to the sub-optimal strategy of avoiding all super-ovipositions at four hosts densities at fly density six. Means and variances are calculated from 10 trials of the Monte Carlo Simulation at each host density.

*** Significant difference at $p < 0.001$;

** Significant difference at $p < 0.01$;

ns No significant difference at $p > 0.05$

Density of Hosts	Optimal Strategy		Sub-optimal Strategy	
	Mean	Variance	Mean	Variance
25	14.83	2.140	7.99	0.001***
100	5.97	0.044	5.55	0.013**
200	3.22	0.004	3.20	0.001ns
300	2.36	0.002	2.37	0.002ns

Analysis of Variance Tables

1 Host density: 25

Source of deviance:	Sums of squares	Degrees of freedom	Mean sums of squares	Fs
Among Strategies	234.10	1	234.10	218.79
Within Strategies	19.28	18	1.07	
Total	253.41			

2 Host density: 100

Source of deviance:	Sums of squares	Degrees of freedom	Mean sums of squares	Fs
Among Strategies	0.89	1	0.89	31.44
Within Strategies	0.51	18	0.028	
Total	1.40			

3 Host density: 200

Source of deviance:	Sums of squares	Degrees of freedom	Mean sums of squares	Fs
Among Strategies	0.0016	1	0.0016	0.65
Within Strategies	0.0444	18	0.0025	
Total	0.0460			

4 Host density: 300

Source of deviance:	Sums of squares	Degrees of freedom	Mean sums of squares	Fs
Among Strategies	0.0005	1	0.0005	0.23
Within Strategies	0.0402	18	0.0022	
Total	0.0402			

8.4 Discussion

Two indicators of population level effects of individual gall fly behaviour have been investigated using the dynamic state variable model: 1) the percentage of hosts which are parasitised, and 2) the mean number of eggs oviposited in hosts. From the results presented in this chapter, the model predicts a number of population level effects which can be related to individual gall fly behaviour.

At a given host density, the percentage of hosts parasitised increases with fly density (Figure 12). The curvature of the function suggests that density dependent processes are at work: as fly density increases, so the increase in the percentage parasitism is less. According to the model, in each time period, the flies in the model population simultaneously search for hosts. Since we are concerned here with percentage parasitism, only encounters with unparasitised hosts need to be considered. Each time a fly oviposits in such a host, the encounter rate of all the flies in the population with unparasitised hosts is reduced, which can be seen as indirect competition for hosts between the flies.

This prediction of the model relates to that of Mangel and Clark (1988, chapter 4, Figure 4.3(b)), in which they plot percentage parasitism against host density for the case in which the ratio of host to flies is held constant. The direct density dependence at low densities is attributed to limited search abilities of the fly. A similar plot is given in Figure 5 for the present system, and shows direct density dependence at low densities, with a flattening out of the curve at high densities.

For a given fly density, the percentage parasitism decreases slightly as the density of hosts increases (Figure 14). The fact that flies are egg limited, particularly at high host densities, accounts for some of the decrease in percentage parasitism. However two features of the model combine to lessen the impact of host density on percentage parasitism. First, the encounter rate with hosts increases with host density. Second, clutch size decreases as encounter rate, and therefore host density, increases, thus offsetting egg limitation and allowing more ovipositions to be made. If clutch size were to be held constant, the percentage parasitism would decrease more rapidly with increasing host density.

In Mangel and Clark (1988, Chapter 4: Figure 4.3(a)), inverse density dependence is predicted for percentage parasitism on host density. The main difference between the Mangel and Clark model and the present one, is in the rate of decrease in percentage parasitism. This difference is due to the fact that variable clutch size is incorporated into the present model, but not into the Mangel and Clark model.

The mean number of eggs oviposited in hosts increases with fly density (Figure 13). At low host densities, super-oviposition is an important factor in increasing the mean number of eggs in hosts. As fly density increases, more super-ovipositions will occur in response to greater percentage parasitism. But as host density increases, super-oviposition becomes less likely. The dependency of clutch size on the distribution of unparasitised compared to parasitised hosts then becomes the dominant feature of the model. As fly density increases, percentage parasitism of hosts increases, and therefore clutch size increases. At a host density of 300, no super-oviposition occurred, and the increase of the mean

numbers of eggs in hosts is attributable solely to variation in clutch size. Furthermore, the difference in slope of the curves in Figure 13 can be related to the likelihood of super-oviposition occurring.

The mean number of eggs oviposited in hosts decreases rapidly at low host densities, and flattens out at higher densities (Figure 15). At low host density, individual flies are more likely to super-oviposit, giving rise to mean numbers of eggs greater than eight, the maximum clutch. Moreover, individuals will oviposit larger clutches at lower host densities. At high host density, super-oviposition is less likely to occur, so the decrease in the mean number of eggs per host is mainly due at higher host densities to the decrease in clutch size.

Mangel (1987) sets out some results of Monte Carlo Simulation of clutch size in relation to host distribution, in which the broad conclusion is that there is a smaller number of large clutches oviposited when the encounter rate with high quality hosts increases. This is equivalent to saying that the mean clutch size is smaller when the density of high quality hosts is greater. In the present model, a high quality host corresponds to an unparasitised host. At high initial host densities, according to the Mangel (1987) model, smaller clutch sizes should be oviposited, which is the case.

By combining both clutch size and super-oviposition choices, and by incorporating changes in host distribution into the same model, the results of the Monte Carlo Simulations do differ in some respects from those of Mangel and Clark (1988) and Mangel (1987), particularly in:

1 The weaker inverse density dependence of percentage parasitism on host density of the present combined model compared to the Mangel and Clark (1988) model.

2 The predictions of egg numbers in hosts greater than the maximum allowed in a single oviposition, because super-oviposition is included in the combined model, but not in the Mangel (1987) model.

8.5 Model predictions and field populations

Comparing the results of the Monte Carlo Simulations of the full optimal model with those of the sub-optimal model in which super-oviposition is always avoided, shows that, at lower host densities, significant reduction of mean numbers of eggs oviposited in hosts occurs with the sub-optimal model. However, if the number of eggs oviposited in hosts exceeds 25, which is the clutch size at which maximum numbers of larvae survive to the gall formation, the point is reached at which super-oviposition becomes a sub-optimal strategy. The model assumes that multiple super-oviposition will occur, and there is no theoretical limit in the model on the number of times super-oviposition will occur in the same host. In the host choice experiment in Chapter 3 multiple oviposition was observed, and in the cage experiment of Chapter 9 very high numbers of gall cells per flowerhead were attained. Multiple super-oviposition was also noted by Varley (1947) in his oviposition experiments. However, in the field, it is unlikely that such multiple super-ovipositions will occur because flies have the option of emigrating from patches (Dempster et al 1995). In order to introduce more realism into the model, emigration and immigration out of and into the patch could be incorporated.

Part 3:
Individual Differences and Population Level Phenomena

Overview

The aims of Part 3 are:

1 To investigate the effect of gall infestation on *Centaurea nigra* plants (Chapter 9).

2 To investigate the population regulation of *Urophora jaceana* in terms of mortality factors acting at different stages of the life cycle (Chapter 10).

3 To relate the within-season distribution of gall infestation and larval mortality to the distribution of plant resources (Chapter 11).

4 To test for selection on plant phentotypic characters resulting from gall-infestation, and to determine whether there is an evolutionary response to selection, by applying heritability tests to the plant phenotypes (Chapter 12).

Chapter 9: Effects of Gall-Infestation on *Centaurea nigra*

Section 1: The effect of gall-infestation on individual plants

The aim of this section is to investigate the impact of gall infestation on individual flowerheads and plants. An experiment is described in which one sample of 18 plants was infested with galls for three consecutive years, and compared to a sample which remained free of infestation for the three years.

9.1.1 Introduction

Seed production is affected by a wide range of factors (Crawley 1983 pp. 52-70), including the rate of pre-dispersal seed predation, and the effects of herbivory. Defoliation of ragwort by cinnabar moth reduces seed production, although there is evidence of plant compensation (Islam and Crawley 1983; Gillman and Crawley 1990). The elimination of sucking insects from broom plants revealed that they cause a considerable reduction in seed production (Waloff and Richards 1977).

In the search for potential agents of biological weed control of thistles and knapweeds introduced into Canada, the weevil *Rhinocyllus conicus*, which destroys seeds of the nodding thistle *Carduus nutans* has been found to be effective (Zwolfer and Harris 1984), although its effectiveness is dependent on the dynamics of seedling recruitment (Crawley 1989). In another biological control study, Myers and Harris

(1980) established that the introduction of two *Urophora* species, *Urophora affinis* and *Urophora quadrifasciata*, into populations of diffuse and spotted knapweed in Canada, led to greater reduction of seed production than that achieved by the gall flies acting singly.

In the same article Myers and Harris (1980) suggest that competition between attacked and unattacked flowerheads on *Centaurea* plants in obtaining metabolic resources will determine the seed production of unattacked heads. In this way, gall infestation will affect not only the seed production of infested heads, but also the seed production of non-infested heads. Galls then act as metabolic sinks (Harris 1980), drawing plant metabolic resources away from non-infested heads.

The presence of galls of *Urophora jaceana* appears to reduce seed production of *Centaurea nigra*. The question is whether it is just the individual infested flowerhead which is affected, or whether the non-infested flowerheads of gall-infested plants also show a reduction in seed production, as in the case cited above. Furthermore, does gall infestation also divert metabolic resources away from other plant functions, thereby altering the allocation of resources to different functions, such as vegetative growth and reproduction, within the plant?

In considering perennial iteroparous plants, like *Centaurea nigra*, there appears to be a trade-off between allocation of resources to vegetative growth and seed production (Kozlowski 1992). More vegetative growth now may be repaid by increased seed production later, whereas greater seed production now may be paid for by reduced seed production later, in response to the need for greater vegetative growth later. If gall infestation uses resources which would otherwise be allocated to

vegetative growth, then gall infestation may not only reduce present seed production, but also future seed production and survival of the plant.

The experiments below are designed to test the following hypotheses:

1 That gall infestation makes no difference to:

- i seed production per flowerhead.
- ii the dry weight of flowerhead material other than seeds and galls per flowerhead.
- iii the total dry weight per flowerhead.

2 That gall infestation makes no difference to:

- i the total seed production of individual plants.
- ii the total dry weight of flowerheads on individual plants.
- iii the dry weight of root material of individual plants.

The tests are carried out using nested analysis of variance for hypotheses 1, and one way analysis of variance for hypotheses 2.

The purpose of testing hypotheses 1.ii, 1.iii and 2.ii is to establish whether or not galls divert plant resources from elsewhere in the plant. The purpose of testing hypothesis 2.iii is to see whether or not galling may have an impact on the vigour or the survival of the plant.

In addition, an estimate of the cost of seed production to the plant per gall is made, in order to be able to estimate the seed production of a gall-infested plant if it did not have galls. This estimate will be used in section 6 in investigating the selection pressure on the plant as a result of galling.

9.1.2 Materials and methods

Seed from one ripe flowerhead of a *Centaurea nigra* plant (from Pontesbury, Shropshire), was collected in early September 1990. In November, the seed was sown in a horticultural seed tray, in commercial coir potting compost (B & Q all purpose), kept moist inside a clear polythene bag, and kept in the light. Germination of most of the seeds took place in the following February 1991. When seedlings had developed four true leaves, a random selection of 36 were transplanted into 4 inch plastic pots containing potting compost. The plants were allowed to grow on outside, watered amply when needed.

The plants were divided into two samples of 18. Each sample was placed inside a fine nylon net cage, 90cm by 90cm by 90cm. Sample 1 was exposed to initially four pairs of gall flies, which were allowed to oviposit in flowerheads, and were replaced as they died. Sample 2 was free of gall flies and acted as a control. Each plant in each sample was numbered. Approximately nine in each sample produced shoots in this first year. The number of galls found in flowerheads of sample 1 was also very low, because bud formation was fairly late, and a number of the gall flies had died before buds were of the right size for oviposition. The net cages were removed before flowering to allow pollination to take place.

The plants were over-wintered under glass, and repotted into 5 inch pots in March 1992, again using B & Q all purpose potting compost from the same bag. The two samples of 18 plants were transferred to the open air, watered as necessary. They were protected from invertebrate herbivores using Murphy's Slug Tape, and by spraying with liquid derris at the first sign of any pest invasion. Just before bud formation, the two samples were placed inside the fine nylon net cages. The number of leaves on each plant at this stage was recorded.

When buds were beginning to develop, a high density of gall flies, in equal numbers of males and females were introduced into the cage of sample 1. The gall flies were those which had emerged from galls collected in the field in 1991, and over-wintered in captivity. They were kept initially in pairs in 2cm glass phials with a drop of honey, before being released.

As gall flies died, fresh ones were introduced, to maintain the high density. The cage of sample 2 was again free of gall flies. The cages were checked daily to ensure gall flies were ovipositing, and to ensure no gall flies escaped into the cage of sample 2. This did happen on one occasion in 1992, when the offending female was removed. It was subsequently found that one plant had been galled in sample 2, and was omitted from the analysis and from subsequent years of the experiment.

Just before the first flowers were beginning to open, and after all gall flies had died, the cages were removed so that pollination could take place naturally. At this stage, the height of each stalk was measured, and each stalk on each plant in both samples was numbered.

Seed heads were allowed to ripen, and were harvested as they became ripe. At harvesting, all seed heads were stored inside paper envelopes.

The process was repeated in 1993 and again in 1994. In March 1993, after the plants in both samples had been over-wintered under glass, they were repotted using fresh coir potting compost into the same pots. In April 1994, rather than repotting, a single slow release plant food tablet (Phostrogen: NPK) was inserted into each pot of both samples.

The seedheads which had been harvested each year of the experiment were dissected and divided into three categories of plant material: seeds, galls and all remaining parts of the seed head (including florets, bracts and receptacle). Seeds were counted.

The wet weight of material of individual seedheads from the 1992 samples was determined. Three subsamples of the three types of material from 30 flowerheads were then dried in an oven at 70^0 C for 12 hours and then weighed. From these weights, conversion coefficients for each type of seedhead material were calculated in order to estimate the dry weights of the whole sample.

All the seedhead material from the 1993 and 1994 samples were oven-dried (12 hours at 70^0 C.) and then weighed. All weights were determined using an electronic chemical balance to the nearest milligramme.

The galls from seedheads of sample 1 were dissected and the number of gall cells in each seedhead determined.

9.1.3 Statistical Analysis

Nested analysis of variance (Sokal and Rohlf 1981) was used to test for significance differences between treatments, in seed production, in total seed dry weight, in receptacle, bract and floret (other seedhead material) dry weight, and in total dry weight per flowerhead. The levels of the nested analysis of variance were individual flowerheads, individual plants, and the two treatments (infested/uninfested) which divide plants into two subgroups. In addition the percentage of the variance contributed by each level was determined.

The nested analysis of variance was carried out using the GLIM programme (Crawley 1993, Sections 8.8 and 14.9). The number and dry weight of seeds were first transformed to the square root in order to approximate better to a normal distribution. The untransformed data for dry weights of other flowerhead material and total dry weight were used. All nested analyses used the identity link function and normal error structure (Crawley 1993). As the samples were of unequal sizes, the procedure for computing average sample sizes and coefficients of variance components, as set out in Box 10.4 of Sokal and Rohlf (1982, Chapter 10) was used, in order to calculate the variance components of the different levels of the nested analysis of variance.

One way Analysis of Variance was used to test for significant differences between treatments in total seed production and total flowerhead dry weight of individual plants. Both annual seed production and total dry weight, and the aggregate over the three years of the experiment were tested.

The seed production of the flowerheads on each plant was summed to give the total annual seed production, and also the total seed production for the three years of the experiment. As these are count data, with a Poisson-like distribution, the Poisson error structure was used with the GLIM programme.

The residual deviance was approximately ten times the residual degrees of freedom in each case, which suggests over-dispersion (Crawley 1993 Section 14.9). In order to take account of the over-dispersion, the scale parameter was adjusted. The value of the scale parameter was calculated by dividing Pearson's X^2 by the residual degrees of freedom (Crawley 1993, Section 14.9).

Refitting the model after adjusting the scale parameter gave residual deviances of the same order of magnitude as the residual degrees of freedom.

The total dry weight of the flowerheads of each plant were also summed in order to calculate the total annual dry weight of flowerheads per plant and the sum of these values gave the total flowerhead dry weight per plant over the three years of the experiment.

The dry weights approximated to a normal distribution in each case, and so the normal error structure was used in the analysis of variance in the GLIM programme.

9.1.4 Results

The wet weights of seed, gall and other seedhead material in gall-infested plants and non-infested plants from 1992 (Table 40) were converted into dry weights, using conversion coefficients for each type of flowerhead material. These conversion coefficients were calculated by dividing the dry weight by the wet weight of similar material from the same seedhead, and by finding the mean of the results for the three samples of 30 (Table 41).

Table 40: Mean wet weights, with standard errors, of seeds, galls and other seedhead material from individual flowerheads and individual plants of gall-infested and non-infested samples for the year 1992. Weights are given in milligrammes.

	Individual seedheads				Individual plants			
Sample	Infested		Non-infested		Infested		Non-infested	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Seed wt	19	1.5	80	5.0	143	5.7	661	86.9
Gall wt	127	6.2	-	-	942	24.9	-	-
Other wt	102	3.0	161	6.5	754	14.7	1336	104.5
Total wt	249	8.7	240	10.5	1838	37.1	1997	180.0
Sample no.	133		141		18		17	

Table 41: Coefficients to convert wet weights to dry weights of seed, gall and other seedhead material, with standard errors.

Seedhead material:	Conversion coefficient	s.e.
Seed	0.978	0.0117
Gall	0.775	0.1369
Other (including florets, bracts, receptacle)	0.969	0.0067

The mean and standard error of seed number, and of the dry weights of each category of seedhead material for the three years of the experiment (Table 42), show that mean seed production and mean seed dry weight of individual plants and of individual flowerheads in the gall infested sample were consistently less than in the non-infested sample. Other plant material dry weight also showed a reduction in the infested sample.

The mean number of galls per flowerhead of 12 in 1994 shows a marked increase over the previous two years (eight and seven respectively) (Table 42). The number of galls per plant shows variation between the three years. However, the level of galling is higher in all three years of the experiment than is found in the field (See Chapter 9).

In 1994, one plant in each of the samples produced no flowerheads. These plants have been omitted from the data presented.

Table 42: Mean seed production and mean dry weights, with standard errors, of seedhead material of infested and non-infested samples for the years 1992, 1993, 1994. Weights are in milligrammes.

1992	Individual seedheads				Individual plants			
Sample	Infested		Non-infested		Infested		Non-infested	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Seed no.	16	2.6	56	2.6	86	15.2	406	54.4
Seed wt	27	3.2	90	5.0	143	26.5	660	86.7
Gall no.	8	0.6	-	-	44	5.5	-	-
Gall wt	136	8.6	-	-	730	89.3	-	-
Other wt	128	3.9	175	5.1	687	64.0	1280	101.3
Total wt	290	9.9	266	8.7	1560	147.2	1939	176.9
Sample no.	97		124		18		17	

Table 42 contd....

1993	Individual seedheads				Individual plants			
Sample	Infested		Non-infested		Infested		Non-infested	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Seed no.	15	2.2	51	2.1	59	17.6	265	35.6
Seed wt	26	4.5	93	4.7	102	27.3	489	67.6
Gall no.	7	0.5	-	-	29	5.6	-	-
Gall wt	136	8.8	-	-	536	98.4	-	-
Other wt	102	50.9	168	46.3	400	71.1	881	112.3
Total wt	263	10.9	262	8.3	1038	176.7	1369	177.2
Sample no.	71		89		18		17	

1994	Individual seedheads				Individual plants			
Sample	Infested		Non-infested		Infested		Non-infested	
	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
Seed no.	12	1.9	59	2.9	58	13.7	225	19.7
Seed wt	21	3.8	112	7.2	103	28.2	427	29.4
Gall no.	12	0.7	-	-	58	7.0	-	-
Gall wt	145	10.8	-	-	708	113.8	-	-
Other wt	134	5.0	173	5.2	656	85.2	661	49.7
Total wt	300	13.5	286	11.0	1467	200.0	1089	72.5
Sample no.	83		61		17		16	

Effect of gall infestation on individual flowerheads

The number of seeds per flowerhead was significantly higher in each year for uninfested plants than for infested plants (Table 43). Similarly, the dry weight of seeds was significantly higher in each year for uninfested plants than for infested plants (Table 44). The null hypothesis (1.i), that gall infestation makes no difference to seed production per flowerhead, must therefore be rejected.

Table 43: Nested Analysis of Variance Tables, to test the effect of gall infestation on seed number per flower head in the three years of the experiment. Seed number was transformed to the square root, and the identity link with the normal error structure in the GLIM programme was used. ** significant at $p < 0.01$.

1992	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var. comp
Among:							
Treatments	908.6	1	908.6	46.8	42.9**	0.15	56.1
Plants	621.2	32	19.4	5.0**		2.48	17.1
Flowers	720.0	185	3.9			3.90	26.8
Total	2249.8	218					100.0

Table 43: contd.....

1993	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	717.0	1	717.0	54.3	46.8**	8.93	65.5
Plants	396.2	30	13.2	5.2**		2.18	15.9
Flowers	319.5	126	2.5			2.54	18.6
Total	1432.7	157					100.0

1994	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	810.0	1	810.0	72.3	67.8**	11.35	63.9
Plants	336.1	30	11.2	2.3**		1.44	8.1
Flowers	530.8	107	5.0			4.96	28.0
Total	1676.8	138					100.0

Table 44: Nested Analysis of Variance Tables, to test the effect of gall infestation on seed dry weight per flower head in the three years of the experiment. The square root of seed dry weights, and the identity link with the normal error structure in the GLIM programme were used. ** significant at $p < 0.01$.

1992	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	1411.0	1	1411.0	49.7	45.8**	12.68	54.1
Plants	908.6	32	28.4	3.8**		3.35	14.3
Flowers	1373.6	185	7.4			7.42	31.6
Total	3693.2	218					100.0

1993	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	1385.0	1	1385.0	64.3	56.4**	17.31	64.6
Plants	646.0	30	21.5	3.4**		3.09	11.5
Flowers	805.2	126	6.4			6.39	23.9
Total	2836.5	157					100.0

Table 44: contd....

1994	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	1574.0	1	1574.0	76.1	71.9**	22.07	63.2
Plants	620.7	30	20.7	2.0 **		2.35	6.7
Flowers	1124.3	107	10.5			10.51	30.1
Total	3318.6	138					100.0

Table 45: Nested Analysis of Variance Tables, to test the effect of gall infestation on the dry weight of florets, bracts and receptacle per flower head in the three years of the experiment. Untransformed dry weights, and the identity link with the normal error structure in the GLIM programme was used. ** significant at $p < 0.01$.

1992	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	130036	1	130036	32.9	31.3**	1156	32.2
Plants	126296	32	3947	1.8**		286	7.9
Flowers	398522	185	2154			2154	59.9
Total	654855	218					100.0

Table 45: contd....

1993	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	194630	1	194630	53.4	46.8**	2423	60.4
Plants	109351	30	3645	3.4**		527	13.1
Flowers	133436	126	1059			1059	26.5
Total	437416	157					100.0

1994	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	59072	1	59072	16.3	15.2**	785	28.9
Plants	108534	30	3618	2.5**		506	18.6
Flowers	152868	107	1429			1429	52.5
Total	320474	138					100.0

Table 46: Nested Analysis of Variance Tables, to test the effect of gall infestation on the total dry weight per flower head in the three years of the experiment. Untransformed dry weights, and the identity link with the normal error structure in the GLIM programme was used. ** significant at p < 0.01; ns not significant.

1992	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	23835	1	23835	0.9	0.8ns	-52	-0.5
Plants	868731	32	27148	4.4**		3343	35.2
Flowers	1146984	185	6200			6200	65.3
Total	2039550	218					100.0

1993	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	754	1	754	0.0	0.0ns	-182	-2.9
Plants	400711	30	13357	2.8**		1755	27.8
Flowers	598278	126	4748			4748	75.1
Total	999744	157					100.0

Table 46: contd....

1994	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	Fs'	Variance component	% Var comp
Among:							
Treatments	1132	1	1132	0.0	0.0ns	-418	-3.4
Plants	843766	30	28126	3.5**		4645	37.9
Flowers	860557	107	8043			8043	65.5
Total	1715646	138					100.0

The dry weight of florets, bracts and receptacles of seedheads for uninfested plants was significantly higher in each year than for uninfested seedheads (Table 45). The null hypotheses (1.ii) that there is no difference in other flowerhead material dry weight per flowerhead between gall infested and non infested plants, must be therefore be rejected in each case.

The total dry weight per flowerhead was not significantly different between infested and non infested plants (Table 46). There are no grounds for rejecting the null hypothesis (1.iii) that there is no difference in total dry weight of flowerheads between gall infested and non infested plants.

Effect of gall infestation on individual plants

Both the annual seed production per plant and the total seed production per plant, summed over the three years, was significantly higher for uninfested plants than for infested plants (Table 47). The null hypothesis (2.i) that gall infestation makes no difference to total seed production per plant can therefore be rejected.

Table 47: Analysis of Deviance Tables, testing the effect of gall-infestation on the sum of seed production per plant for each of the three years of the experiment, and for the sum of the three years. The Poisson error structure and the identity link were used in the GLIM Programme. Over dispersion was adjusted for using a scale parameter calculated from Pearson's X^2. ** significant at $p < 0.01$.

1992	Scaled Deviance	Degrees of freedom	X^2
Among Treatments	38.6	1	38.6**
Within Treatments	36.1	33	
Total	74.7	34	

Scale parameter: 98.6

Table 47: contd....

1993	Scaled Deviance	Degrees of freedom	X^2
Among Treatments	26.6	1	26.6**
Within Treatments	38.0	33	
Total	64.6	34	

Scale parameter: 93.9

1993	Scaled Deviance	Degrees of freedom	X^2
Among Treatments	23.7	1	23.7**
Within Treatments	38.5	33	
Total	62.2	34	

Scale parameter: 61.7

Sum of three years	Scaled Deviance	Degrees of freedom	X^2
Among Treatments	51.8	1	51.8**
Within Treatments	35.2	33	
Total	87.0	34	

Scale parameter: 149.2

Both the annual total dry weight of flowerheads per plant, and the total dry weight of flowerheads per plant, summed over the three years, were not significantly different between infested and uninfested plants (Table 48). There are no grounds for rejecting the null hypothesis (2.ii) that there is no difference in the total dry weight of flowerheads per plant.

Table 48: Analysis of Variance Tables, testing the effect of gall-infestation on the total dry weight of flowerheads per plant for each of the three years of the experiment and for the sum of the three years. Untransformed dry weights with the identity link and normal error structure were used in the GLIM programme. ns not significant.

1992	Sum of squares	Degrees of freedom	Mean Sum of squares	Fs
Among Treatments	1256243	1	1256243	2.58ns
Within Treatments	16066257	33	486856	
Total	17322500	34		

1993	Sum of squares	Degrees of freedom	Mean Sum of squares	Fs
Among Treatments	959542	1	959542	1.65ns
Within Treatments	19200664	33	581838	
Total	20160206	34		

Table 48 contd....

1994	Sum of squares	Degrees of freedom	Mean Sum of squares	Fs
Among Treatments	1559882	1	1559882	3.02ns
Within Treatments	17029646	33	516050	
Total	18589528	34		

Sum of three years	Sum of squares	Degrees of freedom	Mean Sum of squares	Fs
Among Treatments	724936	1	724936	0.29ns
Within Treatments	81823480	33	2479499	
Total	82548416	34		

Effect of the treatment on root dry weight at the end of the experiment

The root dry weight of infested plants was not significantly different to that of uninfested plants (Table 49). There are no grounds for rejecting the null hypothesis that gall infestation makes no difference in root dry weights of plants.

Table 49: Means and standard errors of root dry weight after harvesting in 1994, with analysis of variance table. Identity link and normal error structure.

Infested		Non-infested	
Mean (g)	Standard error	Mean (g)	Standard error
7.12	0.47	6.40	0.39

Analysis of Variance Table

Sum of three years	Sum of squares	Degrees of freedom	Mean Sum of squares	Fs
Among Treatments	4.62	1	4.62	1.37 ns
Within Treatments	114.41	34	3.37	
Total	119.03	35		

9.1.5 Discussion

At the high density of gall infestation of this experiment, seed production of individual flowerheads, and of individual plants is clearly markedly reduced, but the total dry weight of flowerheads is not affected, neither for individual flowerheads nor for individual plants.

Table 50: Summary of results for each null hypothesis

	Null hypothesis:	Accept/reject	Probability
	That there is no difference due to galling on:		
1.a	Seed production per flowerhead.	reject	$p << 0.001$
1.b	Dry weight of other material per flowerhead.	reject	$p << 0.001$
1.c	Total dry weight per flowerhead.	accept	ns
2.a	Total seed production of individual plants.	reject	$p << 0.001$
2.b	Total flowerhead dry weight of individual plants.	accept	ns
2.c	Root dry weight of individual plants.	accept	ns

Approximately a fivefold reduction in seed production was achieved. Size for size, gall infested plants produce significantly less seed than non-infested plants, so their contribution to future generations of seedlings is correspondingly reduced.

The mean seed loss of an individual plant per gall cell can be calculated from the mean seed production of infested and non-infested plants and mean number of galls per infested plant as:

$$S_{LC} = \frac{S_{NP} - S_{IP}}{G}$$

where S_{LC} = mean seed loss per gall cell per plant

S_{IP} = mean seed production of infested plants

S_{NP} = mean seed production of non infested plants

G = mean number of gall cells per plant.

Standard errors can be calculated by adding the standard errors of the mean seed production and dividing by the mean number of gall cells per plant.

In 1992, the mean seed loss per gall cell per plant was 7.25 +/- 1.58, in 1993 it was 7.10 +/- 1.83 and in 1994 it was 2.88 +/- 0.58. How can the discrepancy between the values for 1992 and 1993 and for 1994 be accounted for? In 1994, the level of gall infestation was much higher then in the previous two years, whereas the difference in seed production was no greater. It may be the case that there is a maximum of resources in the plant available to be diverted into gall production.

As gall infestation increases, the maximum is approached and so less resources of the plant are diverted into the production of a single gall cell.

It was found that there was no significant difference in the total dry weight per flowerhead between gall-infested and non-infested plants, a result which was consistent in all three years of the experiment. It would appear, therefore, that galled flowerheads do not divert additional resources to the production of galls. The reduction in dry weight of seeds and of other flowerhead material appears to be sufficient for the production of galls within the the same flowerhead.

There is no difference in total dry weight of flowerheads per plant as a result of galling. The total dry weight of the flowerheads of a plant is a measure of the resources available to the plant for reproduction. It appears that even with heavy gall infestation, the plant is not induced to draw upon any additional resources allocated to other functions, which is confirmed by the comparison of root dry weights at the end of the experiment.

In work on golden rod (*Solidago altissima*) (How, Abrahamson and Zivitz 1994; McCrea and Abrahamson 1985), it was found that galling by the stem gall maker *Eurosta solidaginis* resulted in a greater tendency of rhizomal connections in the roots to disintegrate (sloughing). In this case, oviposition occurs in the stem of the plants, and not in the flowerhead, and the authors suggest that plant hormones may play a part in sloughing.

In the case of *Centaurea nigra*, galling of flowerheads, while significantly reducing the seed production in any year does not seem to have a long-term effect on the plant. Galls do not act as nutrient sinks (Hartley and Lawton 1992) beyond the individual flowerhead, contrary to the suggestion of Myers and Harris (1980), and are not likely to affect the vegetative growth or over-wintering capacity of plants. This conclusion enables seed production to be regarded as the key component of fitness which is affected by gall infestation, and is an important assumption in Chapter 12.

Section 2: The effect of the insect herbivore on plant population dynamics: seed production and seedling density.

In this section the aims are:

1 To compare seed density in a gall-infested population of plants, with that in a population free of gall infestation.

2 To compare the density of germination in the two populations.

3 To compare seedling survival one year after germination in the two populations.

9.2.1 Introduction

In Section 1 of this Chapter it was found that gall infestation of *Centaurea nigra* significantly reduced seed production per flowerhead and per plant. However, galling did not change the total dry weight of flowerhead material. In the field, densities of gall flies are much lower (see Chapter 10) than that used in the experiment, and so it is not clear from that experiment whether seed production in the field is significantly reduced by galling.

A number of studies have found, moreover, that it is often not seed production which affects the population dynamics of a plant, but that it is post dispersal seed herbivory (Hulme 1994), germination success, and seedling mortality, which are the more important factors (Crawley 1989). In the field site described in Chapter 1, it is possible that competition for germination microsites, and mortality of seedlings due to shading by the ground cover, may be the main determinants of plant recruitment.

In the field experiment described below, gall flies were excluded from a 9 m² plot and seed production per flowerhead, germination density, and seedling density one year after germination were compared to a similar plot which had a natural density of gall flies.

The null hypotheses which will be tested are that gall infestation:

1 Does not significantly reduce seed density

2 Does not significantly reduce density of germination

3 Does not significantly reduce the density of seedlings surviving one year after germination.

9.2.2 Method

The experiment was carried out in two 9 m² plots in the field site (see chapter 1). The plots were selected for the similarity of *Centaurea nigra* density and for similarity of aspect. The position of the plots in the field site are shown in Figure 1. One of these plots was chosen as the exclusion plot, from which adult gall flies were excluded, whereas in the other plot there was a natural density of gall flies.

Method of exclusion

In May 1991, and May 1992, all dead seedheads remaining from the previous year, which contained galls, were removed from the plot. There were some seedheads remaining on stalks, but also many which had fallen to the ground. Consequently, the area was searched very thoroughly. The intention was to clear the plot of galls before any adult flies had emerged.

A tent made of fine nylon netting was then erected over the exclusion plot before any adult gall flies were observed in the field. Dimensions of the tent were 3 metres by 3 metres by 1 metre high. The size of mesh (0.5 mm) was smaller than adult gall flies and of all but one of its parasitoids (*Tetrastichus sp B*). Care was taken to ensure that there were no gaps at ground level, and that the entrance to the tent was also secure. The tent was large enough to cover the area of the plot, and high enough to allow the *Centaurea nigra* plants to grow freely.

While the tent was up, it was checked daily for any gall flies which may have gained entry, or which may have emerged from unremoved galls. A number of flies were removed using a pooter as a result of these daily checks.

When no more gall flies were observed in the field, the tent was taken down to allow the plants to continue to grow unimpeded and for flowers to be pollinated. The effectiveness of exclusion in the years 1991 and 1992, expressed as percentage infestation of flowerheads, compared to the percentage infestation of flowerheads in the control plot, is shown in Table 1. The table also shows the period in which the tent was erected in both years. The effectiveness, although not perfect, is adequate for the purposes of testing the hypotheses above.

Data collection: Estimate of seed density

The density of seed produced in both plots was estimated in 1991 and in 1992, in order to compare the seed production in a gall-infested population with that in a population free of gall infestation.

In the middle of August in both years, at the time when all flowerheads are in flower and therefore easily visible, a census was taken of the density of flowerheads in each plot. The number of flowerheads was counted in nine 1 metre by 1 metre quadrats in each plot, therefore covering the whole area of the plots. The density of flowerheads was then compared between the two plots, in order to test whether the two populations were similar.

The number of seeds per flowerhead was taken as the measurement of seed density, so that a direct comparison of the effects of the presence or absence of galls could be made, which is not masked by any differences in flowerhead density.

At the end of August or beginning of September, when seed had set, approximately 10% of the flowerheads in each plot were harvested. Flowerheads were collected evenly over the area, and approximately equal numbers of leading heads and second or third heads down the stem were included. The seed contained in these two samples of flowerheads was counted, flowerhead by flowerhead. The means of the number of seed per flowerhead was then calculated for both samples, and compared using one way analysis of variance.

Census of seedlings

Seeds of *Centaurea nigra* germinate mainly in April, with some germinating in November. A census of seedlings was undertaken at the end of April in 1991, 1992, and 1993.

In April 1991, a preliminary census of newly germinated seedlings was undertaken, in order to provide a comparison of seedling density and seedling survival between the two plots before the exclusion of gall flies was performed. Six 0.5 metre by 0.5 metre quadrats were selected in each plot by using randomly selected co-ordinates, and divided into 25 0.1 metre by 0.1 metre sub-quadrats. The number of seedlings in each sub-quadrat was counted. The corners of the quadrats were permanently marked using metal skewers with plastic tags attached. A proportion of the seedlings were marked by slipping a section of plastic straw round the stem of the seedling. The number of marked seedlings surviving at the end of April 1992 was counted.

In April 1992 both plots were divided into 36 quadrats, 0.5 metres by 0.5 metres. Eighteen regularly-spaced quadrats, in a chequered pattern, were used in the census of seedlings. The number of seedlings in these quadrats was counted and a proportion marked with sections of plastic straw. The number of marked seedlings surviving in April 1993 was counted. The same method was used in the census of seedlings in April 1993 and for survival of marked seedlings in 1994. From these two years of census, a comparison of seedling density and seedling survival between the exclusion plot and the control plot was made, using one way analysis of variance.

9.2.3 Results

Exclusion of *Urophora jaceana* from the exclusion plot is indicated by the percentage of flowerheads in the sample collected in that plot which were infested with galls, for the years of the study (Table 51). After the first year, infestation was very low.

Table 51: Percentage of flowerheads infested with galls in Exclusion Plot compared to natural percentage infestation.

	1991	Tent erected		1992	Tent erected	
		From	To		From	To
Exclusion	19%	13 June	22 July	7%	6 June	13 July
Control	56%			78%		

Density of flowers

The mean densities per 0.25 square metre, and their standard errors, of *Centaurea nigra* flowers in the gall-infested and exclusion plots, were calculated from data gathered in 1991 and 1992. There was no significant difference in flower density between the gall-infested and exclusion plots in 1991 (Table 52).

Seed densities

The mean numbers of seed per flowerhead, with their standard errors, in the samples from both the control and exclusion plots were calculated for 1991, and 1992 (Table 53). Analysis of variance, using the normal error structure, was used to test difference in seed production between the two plots. The number of seeds per flowerhead of the gall infested plot is significantly less than that of the exclusion plot in both 1991 and in 1992. The null hypothesis that there is no difference in seed production between the two plots must be rejected.

Table 52: Mean Density of Flowers (with standard errors) in the two plots, with analysis of variance tables. Analysis of variance performed on untransformed data, using the identity link and normal error structure. ns not significant.

		Gall infested		Exclusion	
		Mean	s.e.	Mean	s.e.
1991ns	per 0.25 m^2	68.55	16.81	71.67	6.30
1992ns	per m^2	130.44	25.58	159.89	15.14

Analysis of Variance Tables

1991	Sum of squares	Degrees of freedom	Mean sum of squares	F_s
Among Plots	44	1	44	0.027 ns
Within Plots	26112	16	1632	
Total	26156	17		

1992	Sum of squares	Degrees of freedom	Mean sum of squares	F_s
Among Plots	3901	1	3901	0.872 ns
Within Plots	71573	16	4473	
Total	75475	17		

Table 53: Mean Numbers of seed per flowerhead (with standard errors) and Analysis of Variance Tables. Analysis of Variance was performed on the untransformed data, using the identity link and the normal error structure.

	Gall infested plot Seeds per flowerhead:			Exclusion plot Seeds per flowerhead			
	Mean	s.e.	n	Mean	s.e.	n	
1991	24.64	1.73	132	38.52	1.95	111	$p < 0.005$
1992	18.56	1.86	82	40.88	2.16	93	$p < 0.005$

Analysis of Variance Tables. ** significant at $p < 0.005$

1991	Sum of squares	Degrees of freedom	Mean sum of squares	F_s
Among Plots	11582	1	11582	28.32 **
Within Plots	8529	241	409	
Total	110111	242		

1991	Sum of squares	Degrees of freedom	Mean sum of squares	F_s
Among Plots	21711	1	21711	59.64 **
Within Plots	62979	173	364	
Total	84690	174		

Table 54: Mean seedling densities and standard errors per 0.5 metre square. ns not significant. The densities for 1991 are before the exclusion experiment.

	Control Plot seedlings		Exclusion plot seedlings	
	At germination		At germination	
	mean	s.e	mean	s.e.
1991ns	55.50	5.59	76.33	10.44
1992ns	12.89	3.40	30.67	8.46

Seedling densities and survival

The mean densities of seedlings of *Centaurea nigra* were calculated from the census of seedlings in 18 0.25 square metre quadrats in both the control and exclusion plots (Table 54).

There was no significant difference in seedling density between the two plots prior to the exclusion of gall flies (Table 55). In 1992, following exclusion of gall flies from one plot in 1991, there was again no significant difference in seedling density between the plot from which gall flies were excluded and that which had a natural density of gall flies (Table 55).

After one year, the density of surviving seedlings which had germinated in 1992 was 0.92 in the non-infested plot and 0.82 in the infested plot. There is no significant difference in survival of seedlings one year after germination between the two plots (Table 55).

Table 55: Analysis of Variance Tables: Seedling densities are square root transformed. Identity link and normal error structure used with GLIM programme.

1991 Seedling Density: before exclusion experiment

	Sum of squares	Degrees of freedom	Mean sum of squares	F_s
Among Plots	4.53	1	4.53	2.73 ns
Within Plots	16.60	10	1.66	
Total	21.13	11		

1992 Seedling Density: one year after exclusion

	Sum of squares	Degrees of freedom	Mean sum of squares	F_s
Among Plots	19.31	1	19.31	2.91 ns
Within Plots	225.69	34	6.64	
Total	245.00	35		

Table 56: 1993 Seedling survival: one year after germination: surviving seedling density square root transformed. The identity link and normal error structure are used with the GLIM programme.

	Sum of squares	Degrees of freedom	Mean sum of squares	F_s
Among Plots	0.38	1	0.39	0.53 ns
Within Plots	25.02	34	0.74	
Total	25.41	35		

9.2.4 Discussion

Gall infestation, at the levels found in the field, does appear to reduce significantly the seed production of *Centaurea nigra*. In 1991 galling significantly reduced the number of seeds per flowerhead to about 64%, and in 1991 to about 46% of seed production in the absence of galls. However, the number of seeds which germinate the following spring is not significantly reduced by galling, in the field site. *Centaurea nigra* does not have a large seed bank. Post dispersal seed herbivory, and competition for germination microsites are therefore likely to be important factors in this scenario. Silvertown and Smith (1989) also found that emergence of *Cirsium vulgare* seedlings in paddocks with different sheep grazing regimes was positively correlated to the level of grazing, and that seed losses between dispersal and germination is a key factor in *Cirsium vulgare* population dynamics. However, de Jong and Klinkhamer (1988) found that emergence of *Cirsium vulgare* seedlings in sand dunes was correlated with seed production the previous year, unlike the present case with *Centaurea nigra*.

The survival of seedlings is also very low in both plots, and there is no significant difference in the number of seedlings surviving one year between the exclusion plot and the plot with natural densities of gall flies in 1993. The effects of shading due to inter- and intra-specific competition (Crawley 1983) appear to be important here.

In terms of overall population dynamics, in a situation where there is:

1 high post dispersal seed herbivory

2 competition for germination sites

3 high seedling mortality

gall infestation at the levels observed in the field, does not appear to have a significant effect on plant recruitment.

However, if individual plants gall are infested at different intensities, then gall infestation should affect the relative reproductive success of individuals, and therefore gene frequency changes. So, while recruitment of plants at the population level is not affected by gall infestation, the gene frequency of the population of individuals may be affected. In Chapter 12 this point is considered further.

»»»»»»»

Chapter 10: The Regulation of Gall Fly Population by Natural Enemies

The overall aim of this section is to investigate some of the potential key factors in the regulation of the gall fly population, and to test for density dependence of these factors.

The main hypotheses to be tested are:

1 That some mortality factors of gall flies act as key factors in gall fly population change.

3 That some mortality factors of gall flies vary with the density of galls, and are therefore density dependent.

Data on the contents of galls, and the density of adult gall flies were gathered from one plot which had a natural density of gall flies. Data were gathered over a period of six years, from 1991 to 1996 inclusive. Four complete years of data were gathered. Life tables were constructed from these data, which compared the killing power of different mortality factors with total killing power.

10.1 Introduction

The analysis of life tables in order to identify those key factors to which the greatest population change may be attributed, has been a standard device used in many studies (Varley 1947, Varley and Gradwell 1960, Harcourt 1971; Redfern and Cameron 1978). A number of methods have been proposed in order to identify key factors. For example, Varley and Gradwell (1960, 1970) suggested a graphical method, in which

the plot of Total Killing Power (k_{Total}) against generation, is compared to the plots of the killing power of the mortality factors (k_i) whose sum equals k_{Total}. The attraction of this method is that it allows for a simple assessment of the relative strengths of the factors. The difficulty with it is that it is rule of thumb, and does not lend itself to significance testing.

A second method (Podoler and Rogers 1975) is to regress each k_i on K_{total}, and to calculate the coefficient of determination. The key factors will be those with highest coefficient of determination and their relative importance will be proportional to the magnitude of their regression coefficients. Although this method appears to be more accurate in assessing the effects of the different mortality factors, the indices which it generates are not susceptible of being tested for statistical significance (Royama 1996). The statistical reason for this difficulty is that the variables being tested are not independent of each other (Begon and Mortimer 1986), and cannot therefore be treated by a conventional multiple regression analysis (Royama 1996).

As long as inappropriate statistical tests are avoided, the method of Podoler and Rogers may be used to give an indication of the relative importance of each factor in contributing to the changes in population size. However, it must be clear as to what is really being analysed here. We cannot speak so much of causes of population change, but more of breaking down population change into constituent parts. A key factor is simply one whose changes from generation to generation are reflected in the overall population changes because of its magnitude in relation to other factors.

Royama (1996) suggests that what may be presented as a single key factor may in fact be a combination of factors. Winter disappearance, which features in many key factor analyses of insect populations, is an example of such a combination of factors. If these factors are defined more accurately and the key factor broken down into its constituent parts, then a different picture of the causes of population change may emerge.

Key factors are not necessarily density dependent (Crawley 1983), and are therefore not necessarily regulators of a population. By definition, the regulation of a population is achieved through density dependent factors. When population increases, a density dependent factor will also increase, and act to reduce the population. By the same token, a density dependent factor will have less impact on a decreasing population, and the effect will be to allow the population to increase. Density dependence can be detected by regressing the killing power of a mortality factor on the logarithm to the base ten of the density of the population immediately before that factor acted (Begon and Mortimer 1986). The coefficient of regression generated in this instance can be subjected to significance testing (Varley et al 1975).

One of the main limitations in the detection of key factors and of density dependent factors in the regulation of populations, is the number of generations for which data are available. Short timescales in relation to the lifespan of the species in question may not reveal potentially important patterns (Crawley 1983), so that for example, a population which appears in a short timescale to be stable, may on a longer timescale be subject to wide variations.

In this section, key factor analysis is applied to data on mortality of gall flies from four consecutive years, in order to identify at which life stages population changes appear to originate. The density dependence of the mortality factors is investigated in order to identify those factors which appear most to regulate the population.

10.2 Methods

Dissection and over-wintering of galls

The galls collected from the harvested flowerheads in 1991, 1992, and 1994, were allowed to over-winter in individual plastic pots, kept in a garden shed. The insects emerging from galls the following spring were identified and recorded. When all insects had emerged, the galls were dissected and the number of gall cells noted.

The galls collected in 1993 and 1995 were dissected in the course of October. Larvae of *Urophora jaceana* and its parasitoids were identified and recorded, as well as the number of gall cells which were empty.

From these data, the percentage of flowerheads infested with galls, and the mean number of galls per flowerhead were calculated, with standard errors. In the same way, the mean number of gall fly larvae, dead or alive, per flowerhead, the mean number of four species of parasitoid larvae per flowerhead, and the mean number of empty gall cells were calculated with their standard errors.

Census of adult flies

Since gall flies emerge over a period of time, and are relatively short-lived, their population density is difficult to assess. Two methods of assessing population density of adult *Urophora jaceana* were attempted. In the spring of 1993, the numbers of the previous year's gall-infested seedheads surviving in the one square metre quadrats were counted. This was repeated again in 1995 and 1996. From the mean numbers of seedheads surviving, the number of gall cells surviving can be estimated from data on the density of galls from the previous year. The number of adult *Urophora jaceana* emerging from these surviving gall cells will then be in the same proportion as adults emerging from the over-wintered sample of gall cells.

The second method, used in 1992, was a capture-mark-release-recapture method. All observed adult *Urophora jaceana* were caught using a pooter, and marked with a small drop of enamel paint, applied with a needle point through a piece of fine nylon netting, to hold the fly still. The marked flies were then released into the plot. Three hours later, all flies observed were again captured and the numbers of marked and unmarked flies were counted. Males and females were distinguished. The number in the population on that particular day was calculated as the proportion of marked flies recaptured multiplied by the total number of flies captured the second time. The method was repeated every six days in the flying season, assuming that a high proportion of flies live no longer than six days (Varley 1947). The density of all gall flies can then be estimated as the sum of numbers in the population on each day that the method was used divided by the area of the study area. This method was used in 1993.

Construction of a life table for *Urophora jaceana*

The data concerning the contents of gall cells and the density of gall flies were used in the construction of a life table for *Urophora jaceana*. The following mortality factors were included in the table:

1 Empty and eaten cells: gall fly larvae eaten by seedhead predators.

2 Parasitism 1: by super-parasitoids which attack both gall fly larvae and the parasitoid *Eurytoma curta*. These include: *Tetrastichus sp. B, Macroneura vesicula, Torymus cyranimus*.

3 Parasitism 2: by the parasitoid *Eurytoma curta*.

4 Other larval mortality, identified by the presence of dead larva in a gall cell.

5 Winter disappearance: mainly by rodent attack of seedheads.

6 Deaths in the pupal state.

By calculating the total killing power of all these factors, and then by regressing the killing power of each factor on total killing power, the key factors of population change were identified (see, for example, Begon and Mortimer 1986, chapter 7).

Identifying density dependent factors

By regressing the killing power of each factor on the logarithm to the base ten of the density immediately before the factor operated, density dependence was identified.

10.3 Results

Gall densities

The density of galls per square metre was calculated as the product of the density of flowerheads per square metre, given in Table 58, and the mean number of gall cells per flowerhead (Table 57). Similarly, the standard error was taken as the product of the standard error of the mean number of gall cells per flowerhead and the density of flowerheads per square metre (Table 57).

Table 57: Percentage infestation, mean number of gall cells per infested flowerhead, and density of galls and flowerheads per square metre. s.e in brackets.

Year	% infestation	Per flowerhead Gall cells		Per m² Gall cells		Flowerheads	
1991	58						
1992	78	4.92	(.44)	500	(45)	130	(25.6)
1993	54	3.63	(.44)	225	(27)	115	(23.1)
1994	53	3.16	(.21)	197	(13)	118	(19.4)
1995	44	3.10	(.23)	170	(13)	124	(18.6)

Contents of galls

Table 58: The contents of dissected gall cells, expressed as mean numbers per infested flowerhead. Standard errors are expressed in brackets. The results of dissecting galls for 1992 and 1994 are based on dissections of over-wintered galls and the record of insects emerged from them. The results for 1993 and 1995 are based on dissections in the autumn of those years, and therefore do not assess pupal deaths. The mean numbers of *Urophora jaceana* for these years are of larvae surviving to 3rd instar. Similarly, the results for 1994 do not include an assessment of larval deaths, which are assumed to be included with empty cells.

	Urophora jaceana		Parasitoids			
			E.c.		T.c.	T.sp B.
1992	1.39	(.22)	0.17	(.06)	0.25 (.07)	0.06
1993	1.80	(.19)	0.27	(.09)	0.14 (.06)	0.05
1994	1.00	(.27)	0.36	(.21)	0.03 (.03)	0.23
1995	1.48	(.20)	0.58	(.15)	0.08 (.04)	0

	Dead Larvae		Dead Pupae		Empty Cells		Number of galls in sample
1992	0.17	(.06)	0.44	(.08)	0.77	(.13)	64
1993	0.12	(.05)			0.33	(.09)	51
1994			0.42	(.11)	1.32	(.28)	31
1995	0.23	(.08)			0.75	(.16)	40

Parasitoid E.c.:	*Eurytoma curta*
Parasitoid T.c.:	*Torymus cyranimus*
Parasitoid T.sp B.:	*Tetrastichus species B*

Densities of adult *Urophora jaceana* in the field.

Adult *Urophora jaceana* densities for 1993 and 1995, are calculated on the assumption that pupal mortality per flowerhead is equivalent to that for the years 1992 and 1994, a mean value of 0.43. Densities range between two or three flies per square metre to 12 flies per square metre (Table 59).

Table 59; Densities of adult *Urophora jaceana* in the field.

1993: gall flies marked-released-recaptured in six day intervals in an area of 20 m^2.

	Marked flies	Recaptured		Estimate
June day		Marked	Total	
24	8	3	10	27
30	15	5	17	51
36	6	2	28	84
42	8	3	12	32
Total estimate				194

Estimate of density: 9.70 flies per m^2

	Mean surviving seedheads (m^2)	Density of pupae of Urophora jaceana	Density of adult Urophora jaceana
1993			9.70 -
1994	5.80 (1.04)	10.44 (1.87)	7.95 (1.42)
1995	2.56 (0.59)	3.66 (0.84)	2.56 (0.59)
1996	11.00 (4.53)	16.28 (6.70)	11.55 (4.76)

10.3 Life Table of *Urophora jaceana*

The Life Table for *Urophora jaceana* is shown in Table 60, where all values are again densities per square metre. The densities of gall cells are as given in Table 1. The mortality attributable to the parasitoids and other factors are calculated from Table 2, by multiplying the mortality per flowerhead due to each factor by the percentage infestation and the density of flowerheads. It is assumed that mortality due to other factors, including predation by other insect larvae, larval and pupal deaths and predation by mice, all act randomly, causing mortality to the parasitoid larvae as well as to the larvae of *Urophora jaceana*. In 1994 it is assumed that the number of dead larvae is included in empty cells. In the life table, the mean value of dead larvae is used and the empty cell value adjusted by subtracting the mean dead larvae value. The number of dead pupae for 1993 and 1995 are assumed to be the mean of the years 1992 and 1994.

Table 60: Life Table of *Urophora jaceana*. The number of gall cells is given as density m^{-2}. The mortality due to over-wintering deaths, in bold type, is deduced from the survival of gall cells the following spring.

Cause of mortality	Mortality	% Mortality	Survival	k	k total
Number of gall cells in 1992			500.00		
Eaten by lepidoptera	78.34	15.67	421.66	0.07	
Attacked by parasitoids	31.54	7.48	390.12	0.03	
Attacked by *Eurytoma curta*	17.30	4.43	372.82	0.02	
Other larval deaths	17.30	4.64	355.52	0.02	
Over-wintering deaths	337.77	95.01	17.75	1.30	
Pupal deaths	8.05	45.35	9.70	0.26	1.71
1993 Adult density			9.70		
Number of gall cells in 1993			225.00		
Eaten by lepidoptera	20.43	9.08	204.57	0.04	
Attacked by parasitoids	11.77	5.75	192.80	0.03	
Attacked by *Eurytoma curta*	16.72	8.67	176.08	0.04	
Other larval deaths	7.43	4.22	168.65	0.02	
Over-wintering deaths	158.21	93.81	10.44	1.21	
Pupal deaths	2.49	23.85	7.95	0.12	1.45

Cause of mortality	Mortality	% Mortality	Survival	k	k total
1994 Adult density			7.95		
Number of gall cells in 1994			197.00		
Eaten by lepidoptera	71.65	36.37	125.35	0.20	
Attacked by parasitoids	16.20	12.92	109.15	0.06	
Attacked by *Eurytoma curta*	22.43	20.55	86.72	0.10	
Other larval deaths	10.59	12.21	76.12	0.06	
Over-wintering deaths	72.46	95.19	3.66	1.32	
Pupal deaths	1.10	30.05	2.56	0.16	1.89
1995 Adult density			2.56		
Number of gall cells in 1995			170.00		
Eaten by lepidoptera	41.03	24.13	128.97	0.12	
Attacked by parasitoids	4.38	3.39	124.59	0.01	
Attacked by *Eurytoma curta*	31.73	25.47	92.87	0.13	
Other larval deaths	12.58	13.55	80.28	0.06	
Over-wintering deaths	64.00	79.72	16.28	0.69	
Pupal deaths	4.73	29.05	11.55	0.15	1.17
1996 Adult density			11.55		

Figure 16:
Killing power of mortality factors compared to k total I.

Mortality Factors: Eaten larvae and larval deaths.

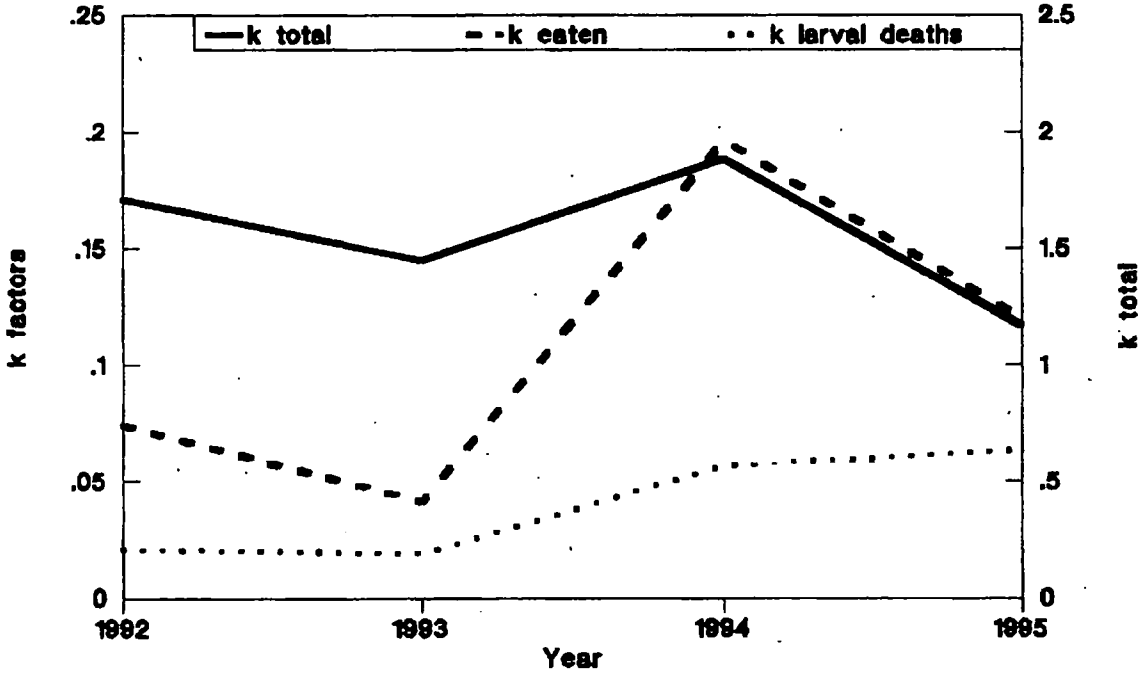


Figure 17:
Killing power of mortality factors compared to k total II.

Mortality Factors: Eurytoma curta and super parasitoids.

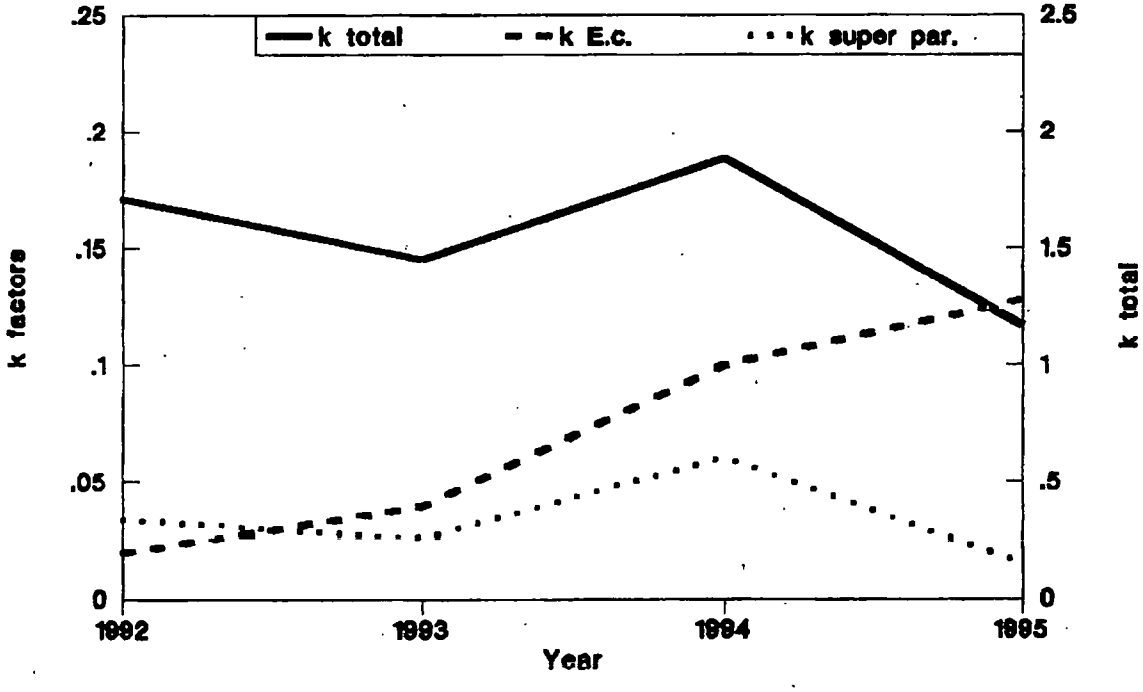


Figure 18:
Killing power of mortality factors compared to k total III.

Mortality factor: Winter disappearance.

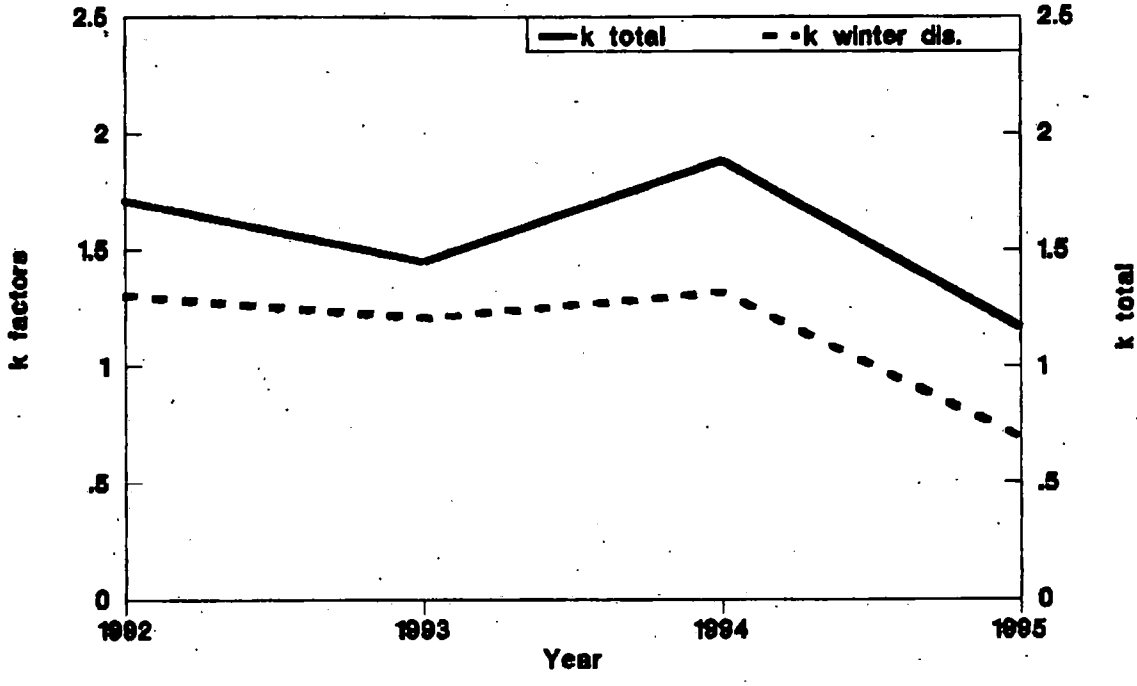
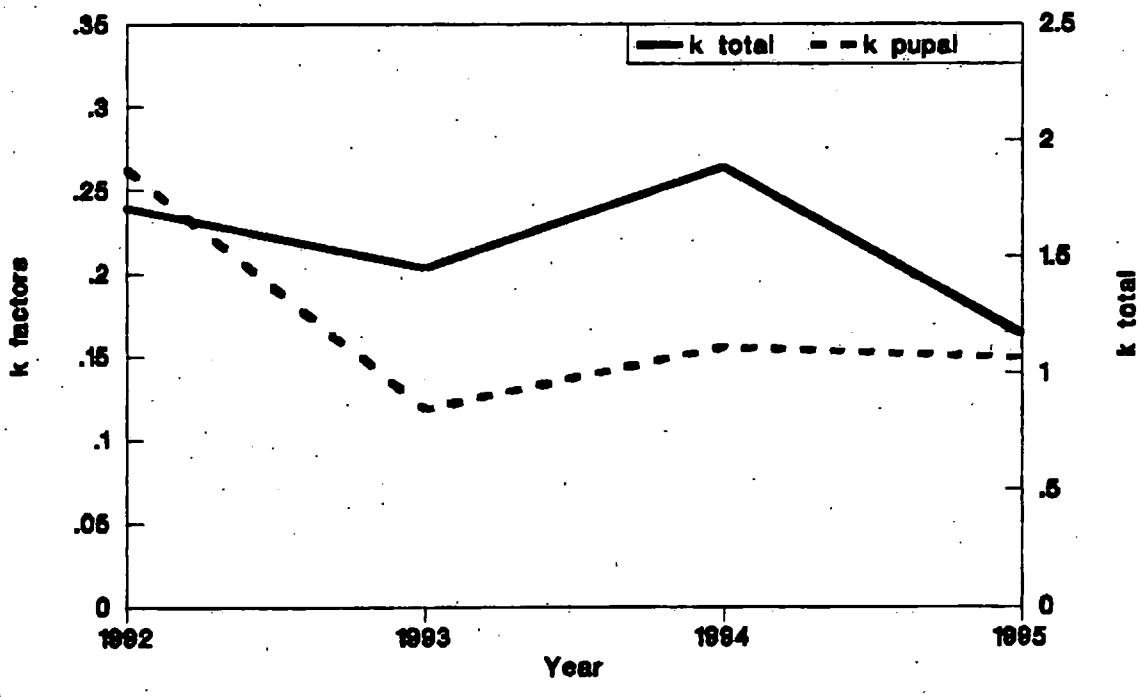


Figure 19:
Killing power of mortality factors compared to k total IV.

Mortality Factor: pupal deaths.



The number of empty gall cells in the sample is assumed to indicate the mortality caused by predation by other insect larvae, including lepidoptera and gall midge larvae. The density of mortality in all these cases was calculated as the proportion of empty cells, or dead larvae, or dead pupae, in the sample multiplied by the density of *Urophora jaceana* larvae surviving after the action of the previous mortality factor in the table.

Mortality attributable to predation by mice, and other "winter disappearance", is the largest factor of all, and is calculated by subtracting the density of the pupal population of *Urophora jaceana* in the field from the density of *Urophora jaceana* larvae before the action of this factor.

The total killing power, and the killing power attributable to each of the factors, is shown plotted against the year in Figures 16, 17, 18, and 19. The plot of killing power due to cell contents being super-parasitised (Figure 17) follows a similar shape to total killing power. The plots of killing power attributable to cell contents being eaten (Figure 16), being parasitised by *E. curta* (Figure 17), to larval deaths (Figure 16) and to pupal deaths (Figure 19) do not follow so closely the pattern of total killing power. All these factors are relatively weak, less than 10% of total killing power. However, killing power attributable to winter disappearance is both the largest factor, and approximates to the pattern of total killing power (Figure (18).

Regressing the killing power of the mortality factors on total killing power gives the results shown in Table 61. The single largest slope by far is that of mortality caused by mice (0.846). The other candidate as a

key factor, super-parasitism, has a low value for slope (0.057). All other mortality factors are shown to have low values for the slope. In view of the difficulties of statistical testing of regression between interdependent variables, it is not possible to say any more than that the only candidate to be a key factor is over-wintering mortality.

Table 61: Regression of k_i on K_{total}.

Mortality factor	slope (b)	S.E. of b	r^2
Eaten	0.090	0.137	0.18
Super-parasitoids	0.057	0.015	0.88
Eurytoma curta	-0.057	0.107	0.12
Larval deaths	-0.013	0.052	0.03
Over-wintering	0.846	0.294	0.80
Pupal deaths	0.077	0.131	0.15

Density Dependence of mortality factors

Density dependence of mortality factors was tested by regressing the killing power of each mortality factor on the logarithm to the base ten of the density before the effect of that factor. As can be seen in Table 62, none of the mortality factors appears to be density dependent. Indeed the slopes of the first four are negative, which would suggest inverse density dependence. The two highest F-ratios are for *Eurytoma curta* and for larval deaths, but as the slopes in these cases are relatively low, it is unlikely that they have any destabilising effects on the gall fly population.

Table 62: Density dependence of mortality factors.

Regression of killing power on log(density before operation of factor)

Mortality factor	slope (b)	S.E. of b	F-ratio	r^2	
Eaten	-0.139	0.203	0.47	0.19	ns
Super-parasitoids	-0.010	0.055	0.03	0.01	ns
Eurytoma curta	-0.181	0.068	7.15	0.78	ns
Larval deaths	-0.069	0.028	6.17	0.75	ns
Over-wintering	0.439	0.582	0.57	0.22	ns
Pupal deaths	0.081	0.129	0.39	0.16	ns

10.4 Discussion

Key factor analysis of data from short time series is often inconclusive, (Redfern and Cameron 1978; Redfern, Jones and Hassell 1992) and the analysis presented here is no exception. A number of studies have been done in which density dependence within a generation has been studied using patches with different densities of organisms (Cappuccino 1992, Dempster, Atkinson and Cheesman 1995a). In these cases also the basis of population regulation has proved hard to establish. Hassell (1985) suggests that spatial patterns within patches need also to be considered in detecting density dependence within generations. The spatial variation

in density within an area can obscure density dependence, if a population study is not undertaken on an appropriate spacial scale (Ray and Hastings 1996).

In the present case, all mortality factors except over-wintering mortality (mainly destruction of flowerheads by mice) have relatively small effects on the gall fly population, and are therefore unlikely to act as key factors in causing population change. Over-wintering mortality does have larger effects on gall fly population and appears to vary with total killing power, although the correlation is not significant.

These results concur with those of Dempster, Atkinson and Cheesman (1995a) who found that mortality factors acting between oviposition and fully grown larvae had small effects on population change and a non-significant correlation with the total killing power. In contrast, factors operating between larvae being full grown and adult, including fecundity, were found to have much larger effects on the population, and also to approach significance in their correlation to total killing power.

Density dependence of mortality factors was not discovered in the present case, in any stage between gall formation and emergence of adults. Although the method of identifying density dependence used here is different to that used by Dempster, Atkinson and Cheesman (1995a), who applied a spatial, rather than temporal, regression model, nevertheless the conclusions arrived at are again broadly similar. They found that the action of parasitoids was not density dependent. In this respect, the conclusion of Varley (1947), that the parasitoid *Eurytoma curta* was a density dependent factor which regulates gall fly population

is challenged. However, in support of Varley, the population levels of *Eurytoma curta* in the Warmington site are very low. It may be possible that in a longer term study, higher density of the parasitoids may in fact be responsible for population regulation of the gall fly.

Dempster, Atkinson and Cheesman (1995a) concluded that the density of flowerheads was a major factor in population regulation, especially in terms of the competition between adult flies for oviposition sites. High densities of flies, and low densities of flowerheads available for oviposition leads to emigration from one patch to another (Dempster, Atkinson and French 1995b). In the Warmington site, flowerhead density remained fairly constant over the years of the investigation. However, the availability of resources, in this case flowerheads suitable for oviposition, is an important factor in population regulation, and is considered in detail in the next Chapter.

Varley (1947) also considered mortality of gall fly larvae in the stage of development before gall formation to be density dependent, but insufficient on its own to regulate the gall fly population. In Chapter 6 above, it was also found that egg mortality is dependent on the number of eggs laid in a flowerhead. Survival of larvae up to gall formation appears to approximate to a quadratic function of clutch size. Although important in affecting oviposition choices, especially concerning clutch size, this factor is relatively weak at low densities of eggs per flowerhead compared to the overall mortality in all life stages. At high densities of eggs per flowerhead however, there may be a different story (Myers and Harris 1980). High densities will occur as a result of successive super-oviposition, which will happen in certain circumstances; ie low flowerhead density, and high fly density.

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Chapter 11: The Within-Season Variation of Resource Availability, Gall Density and Larval Survival

In this chapter the importance of variation of time of budding of the host plant for the utilisation of available oviposition sites by the insect is considered.

The distribution of the timing of flowerbuds of the host plant becoming available for oviposition is compared to the timing of emergence of adult gall flies within the season. The density of gall cells per flowerhead, and the proportion of gall fly larvae surviving per flowerhead is compared with the time at which flowerheads become available for oviposition.

11.1 Introduction

Dempster and Pollard (1981) have argued that fluctuations in resource availability are an important factor in determining the fluctuations in insect populations. The concept of a population equilibrium regulated by natural enemies (see for example, Varley and Gradwell, 1968) has tended to ignore the availability of resources as they relate to the precise needs of the insect (Dempster and Pollard, 1981). However, examples from a few studies, such as on cinnabar moth and ragwort, and on its parasitoid *Apanteles popularis* (Hal.) (Dempster 1979, 1981), suggest that resources, limited by rainfall in the case of ragwort, or by the inefficiency of *Apanteles* searching for cinnabar moth hosts, do determine population levels.

Straw (1991), studying two flowerhead-attacking tephritid flies which are highly specific in their choice of oviposition sites, noted that both *Cerajocera tussilaginis* and *Tephritis bardannae* oviposit in only a small percentage of lesser burdock (*Arctium minus*) capitula. He suggests that fly density does not affect the percentage attack, but only the number of eggs laid in each attacked flowerhead, and that therefore resource availability or quality, rather than fly density, determines attack rate (Straw 1991). He concludes that, since flowerheads are suitable for attack only at a certain stage of development (Straw 1989a), the long search time needed by the flies in locating suitable oviposition sites effectively limited their exploitation of resources (Straw 1991).

A number of studies (for example Varley and Gradwell, 1968; Hassell, 1969, Rodriquez et al 1994)) indicate that temporal asynchrony between insects and their hosts may be a feature of these interactions. Incorporating the timing of phenological stages into a host-parasitoid interaction reveals that asynchrony may on its own stabilise an otherwise unstable interaction (Godfray, Hassell and Holt, 1994; Iwasa and Levin, 1995).

In the interaction between *Urophora jaceana* and its plant host, oviposition takes place at an early stage of flowerhead development (Varley 1947, and see Chapter 5). As in the case of *Arctium minor*, oviposition sites have limited availability. *Centaurea nigra* has significant variation of flowering time between individual plants (Chapter 2) and it is therefore possible that variation of the timing of buds becoming available for oviposition may lead to 1) fluctuations in the availability of oviposition sites within a season, and 2) relative release of some flowerbuds from infestation.

Here, the frequency distribution of the day of budding of flowerbuds is compared to the frequency distribution of gall fly emergence, and of the emergence of the main parasitoid of gall flies, *Eurytoma curta*. This frequency distribution raises two questions concerning the distribution of galls and the survivorship of gall fly larvae in relation to the density of buds available for oviposition through time within the season:

1 Is a higher proportion of earlier-emerging flower buds gall-infested than later-emerging flowerbuds, and are larger clutches laid in flowerbuds which emerge earlier than later?

2 Are a higher proportion of gall fly larvae attacked by *Eurytoma curta* in later-emerging flower buds than in earlier-emerging flower buds?

11.2 Methods

Frequency distribution of budding times

A three metre by three metre plot in the field site was marked out into one metre square quadrats in May 1995, before any flowerbuds had emerged. From the beginning of June, the plot was inspected at three-day intervals. Every flowerbud which had emerged in the previous three days was counted by quadrat, and marked using sticky paper labels, with the day on which the flowerbud was counted written on them. The purpose of marking flowerbuds was to ensure that flowerbuds were not counted twice, and also to enable identification, later in the season, on which day each bud had emerged. Counting began on 20th June, when

the first bud had emerged, and ended on 14th July, by which time all buds had emerged in the plot. From these data a frequency distribution of the buds emerging in three-day intervals in the whole plot was constructed.

Frequency distribution of insect emergence times

Using the plot in the field site described above, all galls which survived in the plot from the previous year were collected in May 1995, quadrat by quadrat. Galls from each quadrat were put into a 2cm diameter clear plastic pot with a screw top, which was placed in an open glass jar for protection. Each jar was laid on its side on the ground in the quadrat in which the galls were collected. The purpose of putting the galls in pots was to be able to note all insects which emerged from them. By keeping the pots in the field, insects could be released into the field from the same area in which they would have naturally emerged. Pots were then inspected daily. Any insect emerging from galls within the pots was identified in the field, and released into the quadrat in which the pot was placed. From these data, frequency distributions of the day of emergence of gall flies and of *Eurytoma curta* were constructed.

Collection of galls from flowerheads of known day of budding

Ninety flowerbuds from twelve plants in the field site were marked in the middle of June 1992, using sticky paper labels, with a reference number for each flowerbud. Buds were inspected twice weekly, in alternating three- and four-day intervals. As buds emerged the day of emergence was noted.

In September, when all buds had flowered and set seed, they were harvested with identifying sticky labels still attached to the upper stem. Flowerheads were dissected, and the presence of galls noted. As these galls were left to over-winter in order for gall flies to emerge (see Chapter 4 section 2), the galls were not dissected until the following July. The number of gall cells in each gall was noted.

The number of galls per flowerhead was then fitted against the day of budding of the flowerhead in which galls were present, using the GLIM programme.

In 1995, flowerheads were harvested from among those which had been marked on the day of budding within the three metre by three metre plot as described in section 4 of this chapter. As in 1992, flowerheads were harvested after flowering and seed set in September. The aim in harvesting was to obtain a reasonably sized sample of galls in each day of budding class. However, there were very few early buds, so the sample size of the earliest budding classes are small.

Galls were dissected in October according to day of budding class of host bud. The number of gall cells was noted. The following contents of galls were also noted:

1 Number of live gall fly larvae present.

2 Number of dead gall fly larvae.

3 Number of empty cells.

4 Number of cells attacked by *Eurytoma curta*.

5 Number of cells attacked by other parasitoids.

Using GLIM, The following tests were applied to the data:

1 Whether there is a difference in the number of gall cells in flowerheads of different budding time classes.
2 Whether there is a difference in the survival of gall larvae in different budding time classes.
3 Whether there is a difference in the rate of attack of gall fly larvae by *Eurytoma curta* in different budding time classes.

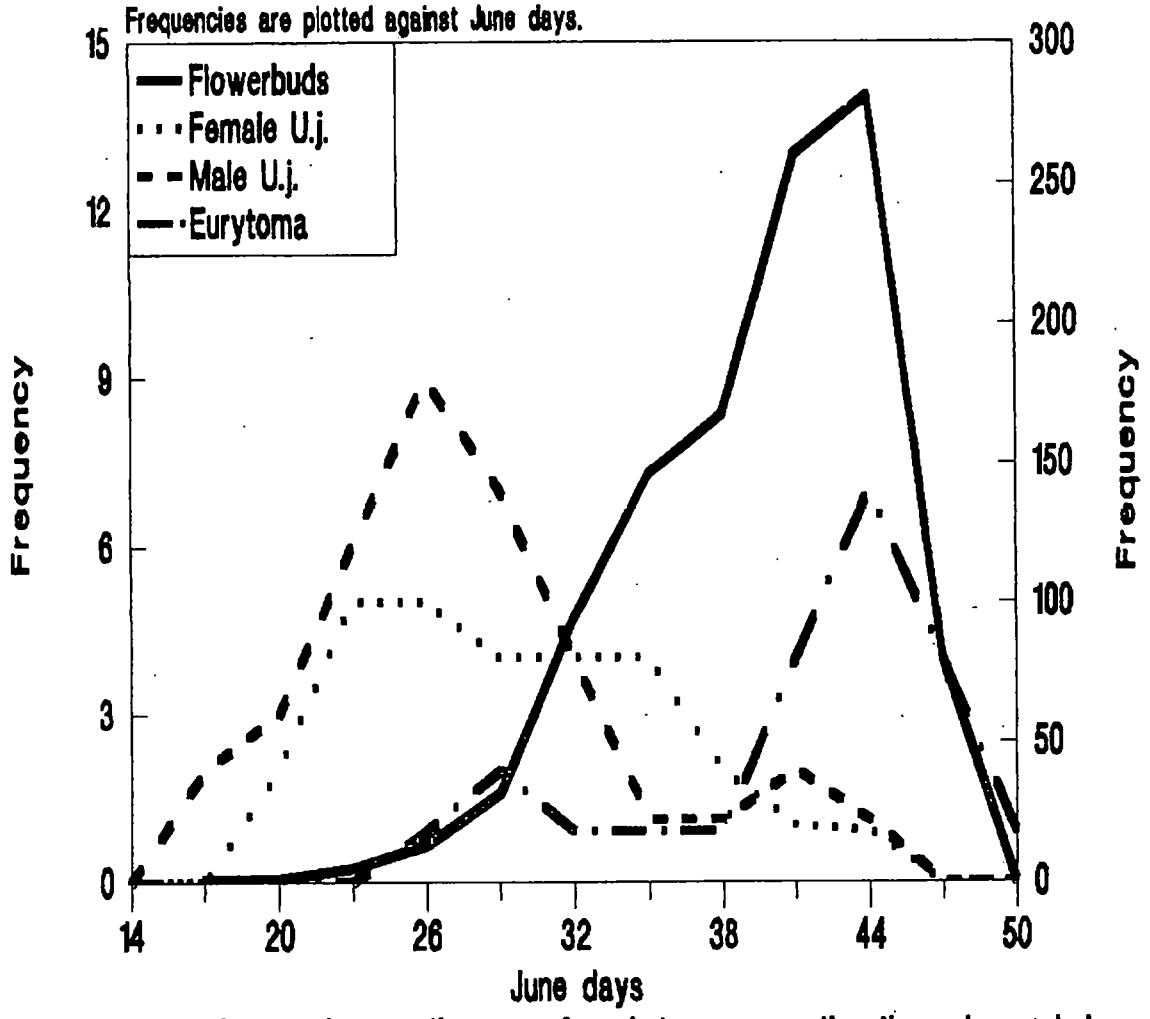
11.3 Results

The frequency of the number of flowerbuds emerging in three day intervals (Figure 20) rises slowly at first and then more rapidly after day 29. On day 44 the number of buds rose to its highest level, coinciding with the day on which all buds had emerged. The frequency of gall flies emerging rises rapidly up to day 23 and then falls more gradually to day 44 (Figure 20). The peak of gall emergence occurs at a time when bud frequency is at the level of five buds, and begins to fall as bud frequency is increasing more rapidly.

No *Eurytoma curta* emerged before day 26 (Figure 10). Between day 26 and day 38 at most one parasitoid emerged each day. On days 41 and 44, peak emergence of *Eurytoma curta* occurred, followed by rapid decline.

The square root of the number of galls per flowerhead in 1992 is inversely proportional to the day of budding, with an F-ratio of 8.41, which is significant at $p < 0.01$ (Table 63). Similarly, there is significant inverse dependence of the square root of the number of galls per

Figure 20:
Frequencies of 1) flowerbuds, 2) female Urophora jaceana, 3) male Urophora jaceana, 4) Eurytoma curta in a 3m x 3m plot in the field site in 1995.
Frequencies are plotted against June days.



Insect frequencies are the sum of each two consecutive three day periods to give an indication of number of insects alive assuming a lifespan of six days.

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flowerhead in 1995 (Table 63). The day of emergence is shown in three-day intervals beginning on 17th June, and the numbers shown are the sums a) of flowerbuds emerging that day; b) of gall flies emerging from galls that day and on the previous two days; and c) of *Eurytoma curta* emerging from galls that day and on the previous two days, in nine 1m² quadrats. The days of emergence are counted from 1st June 1995.

Table 63: 1)Number of galls per flowerhead against day of budding: 1992

The number of galls in each flowerhead was transformed to the square root, and then fitted against the day of budding of the flowerhead using GLIM, using the normal error structure and the identity link. ** significant at $p < 0.01$.

Variable	Error Structure	Intercept	Slope	Fs
√Galls	Normal	3.748	-0.0685	8.41**
		+- 0.57	+- 0.024	

Analysis of Variance Table

	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	
Among day of bud.	4.549	1	4.549	8.41	$p < 0.01$
Error	30.312	56	0.541		
Total	34.861	57			

2) Fitting gall cell data to day of budding: 1995

The number of galls per flowerhead is transformed to the square root, and then fitted against the day of budding of the flowerhead using GLIM, using the normal error structure and the identity link.

Proportional data are analysed with the binomial model, and are transformed into logits, which is equivalent to the natural logarithm of the odds on the event occurring. In each test, overdispersion was detected, which was taken into account using the scale directive, with the scale parameter estimated by dividing the Pearson X^2 value by the residual degrees of freedom. The change in deviance divided by the scale parameter gives the Fs value.

* significant at $p < 0.05$; ** significant at $p < 0.01$; ns not significant

Variable	Error Structure	Intercept	Slope	Fs
√Galls	Normal	2.556 +- 0.273	-0.0221 +- 0.008	8.426 **
Larval survival	Binomial	1.894 +- 0.71	-0.053 +- 0.020	3.475 ns

Table 63: contd....

Proportion cells empty	Binomial	-0.873 +- 0.77	-0.0044 +- 0.022	0.018 ns
Proportion attacked by *Eurytoma curta*	Binomial	-5.985 +- 1.42	0.105 +- 0.038	4.202 *

Analysis of Variance Table: √number of galls per flowerhead

√galls	Sum of squares	Degrees of freedom	Mean sum of squares	Fs
Among day of b.	1.47	1	1.4720	8.426 **
Error	15.55	89	0.1747	
Total	17.02	90		

Analysis of deviance tables

Model:	Deviance	Degrees of freedom	Fs
Larval survival: Corrected for over-dispersion			
Among day of budding	5.00	1	3.48 ns
Error	117.38	89	
Total	122.38	90	

Scale parameter: 1.44

Table 63 contd....

Attack by *Eurytoma curta*: Corrected for over-dispersion				
Among day of budding	6.02	1	4.20	*
Error	76.87	89		
Total	82.89	90		
Scale parameter: 1.43				
Empty cells: Corrected for over-dispersion				
Among day of budding	0.03	1	0.02	ns
Error	104.61	89		
Total	104.64	90		
Scale parameter: 1.52				

The proportion of gall fly larvae surviving is shown to be independent of day of budding in 1995 (Table 63), whereas the proportion of cells attacked by *Eurytoma curta* is directly dependent on the day of budding (Table 63) at $p < 0.05$. The proportion of cells which are empty is independent of the day of budding (Table 63).

11.4 Discussion

The distribution of the number of buds available for oviposition by gall flies, as described above, shows that in the earlier part of the budding season, buds are in short supply. For a period of nine days after the emergence of the first gall flies, the number of new buds becoming

available for oviposition in each three day period in the 9 m^2 plot was less than 32. In this same period, the number of gall flies emerging increased from one to eight per three-day period. If, as recorded by Varley (1947), females do not oviposit for the first three days after eclosion, then it appears that the number of gall flies ready to oviposit reaches a peak just as the number of flowerbuds is about to increase rapidly. After this time, the number of gall flies coming on stream, ready for oviposition, falls slowly, and the number of buds available increases rapidly. It would be reasonable to suggest that the number of eggs oviposited per flowerhead decreases steadily in this period.

The result of fitting the number of galls per flowerhead against the day of budding of those flowerheads bears out this prediction. In 1992, the square root of the number of galls per infested flowerhead decreased by 0.0685 per day, which is significant at $p < 0.01$, and in 1995 by 0.0221, significant at $p < 0.01$.

Predation by flowerhead lepidopteran larvae is independent of day of budding, whereas attack by *Eurytoma curta* increases directly and significantly with day of budding, at $p < 0.01$. This result can be explained in terms of the frequency distribution of the emergence of *Eurytoma curta*. Galls which are formed earlier will have a greater chance of escaping parasitism, compared to those which are formed later.

Iwasa and Levin (1995) discuss the timing of life history events in contributing to the stabilisation of populations. They suggest that in some populations asynchronous reproduction may be a stable strategy given the right circumstances. For example there may be advantages to reproducing early, for example, in terms of less competition for food

supply, or escape from predation, but there will also be risks, for example, due to disturbance, so that there will be a trade-off between early timing and risk of disturbance, and between later timing and lower survival of offspring. In this situation, it would be destabilising for the population if all individuals took advantage of early timing, because they would all be open to potentially catastrophic risks. At the same time, if early timing is successful it will lead to a selection advantage over the later strategy.

In the case of gall flies, there is an advantage in early oviposition, because early galls are more likely to escape attack by *Eurytoma curta*. However, there are also risks to those gall flies which do emerge early, because of the low density of suitable buds in which to oviposit early in the season, resulting in a high level of competition for oviposition sites.

Dempster et al (1995a) conclude that the population regulation of *Urophora jaceana* is due to limiting resources in the adult phase, which leads to emigration from patches where there is a high density of gall flies and low density of flowerheads suitable for oviposition. They suggest, from their own data concerning the distribution of flowerbuds suitable for oviposition during the oviposition period of gall flies in two patches of *Centaurea nigra* that many potentially suitable flowerheads were not usable by gall flies. This conclusion is supported by the data presented here.

In the distributions for 1995 given in Figure 20 above, it is noted that densities of suitable flowerbuds early in the season are very low. At this time gall flies were also beginning to emerge. In this period, competition between gall flies will be at its most intense, and also the

foraging success will be at its lowest. In these conditions, the scenario postulated by Dempster et al (1995a) would seem to apply, in which gall flies choose to emigrate to other patches. They suggest that the rate of emigration depends inversely on the density of flies emerging from a patch.

The movements of gall flies have been investigated using heavy metal marking by Dempster, Atkinson and French (1995b), who concluded that gall flies are very mobile. Distances of at least 800m from the point of emergence have been recorded, and it is possible that even greater distances are travelled in the search for new patches. They suggest that *Urophora jaceana* needs to be studied as a metapopulation, in which local extinctions, emigration from and colonisation of patches are central features. The results presented here suggest that the dynamics of emigration can be better understood by considering the within-season variation in the timing of buds available for oviposition, in relation to the distribution of time of emergence of gall flies.

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Chapter 12: Phenotypic variation and selection of *Centaurea nigra*

Section 1: The measurement of selection on characters of *Centaurea nigra* due to gall infestation by *Urophora jaceana*.

The aim of this section is to test whether gall infestation exerts a selection pressure on plant flowerhead phenotypic characters. Stabilising and directional selection on the phenotypic characters defined in Section 1 of this chapter are measured using the multiple quadratic regression method as described by Lande and Arnold (1983). The size of plants, as measured by the number of flowerheads is also included.

A sample of 30 plants in the field with natural levels of gall infestation was used. The component of relative fitness on which the phenotypes are regressed is annual seed production. The regression using actual seed production of the gall-infested plants is compared with that using an estimate of the seed production that there would have been without gall infestation. Significant differences between the coefficients of the two regressions can then be attributed to gall infestation.

12.1.1 Introduction

Lande and Arnold (1983), following Haldane (1954) and Falconer (1981), make the important distinction between natural selection acting on phenotypes, and the evolutionary response to selection which depends

on genotypic variation (Fisher 1958). Consequently, the measurement of selection on phenotypes can be performed by assessing the contribution that organisms with certain phenotypes make to future generations.

Haldane (1954) defined the intensity of selection, I_H, by comparing the phenotype which had the highest fitness with the mean fitness of the population. The intensity of selection is then given by:

$$I_H = \ln w_0 - \ln w \qquad 1$$

where w_0 is the fitness of the optimal phenotype, and w is the mean fitness. Van Valen (1965) proposed that the intensity of selection be defined as:

$$I = (w_0 - w)/w_0 \qquad 2$$

which can be understood as the proportion of deaths that occur as a result of the mean phenotypic value being less than the optimal value.

The disadvantage of these measures of selection is that the optimal phenotype may or may not exist, and if it does, may not be measurable in practice. O'Donald (1970) proposed a way of overcoming this difficulty by applying Fisher's Fundamental Theorem of Natural Selection (Fisher 1958) to phenotypic selection. He suggested that the proportionate increase in fitness should be used to measure selection, so that:

$$(w'-w)/w = V_w/w^2 \qquad 3$$

where w' is the mean fitness after selection, w is the mean fitness before selection, and V_w is the variance in fitness before selection. This method of assessing the intensity of selection was tested by O'Donald using Bumpus' data (O'Donald 1973, see also Johnston, Niles et al 1972).

O'Donald (1973) suggested that models which describe the change in fitness with deviation of a phenotype from an optimum value can be fitted to data, and that if the proportion of each phenotype in a population before and after selection are known, then relative fitnesses can be calculated directly, and the intensity of selection can be calculated from these relative fitnesses.

The proportionate increase in fitness is dependent on the time interval before and after selection. Manly (1975) suggested an index of selection calculated from an estimate of relative susceptibilities to selection, in order to avoid making the index dependent on the time interval before and after selection. Manly (1977) also suggested an index of selection which employs a double exponential function for the fitness of phenotypic characters.

For plant and animal breeders a key concept is the response to selection (Falconer 1981, chapter 11), which is the difference of the mean phenotypic value of the offspring of parents of a certain phenotypic value from the mean phenotypic value of the parental generation. The selection differential, S, can then be calculated from the slope of the regression of mean phenotypic values of offspring on mid parent, b, and the response to selection R:

$$R = bS \quad \text{which is equivalent to} \quad R = h^2S$$

Robertson (1966) and Price (1970) suggest a method of using the coefficient of regression of relative fitness on a character to measure the selection gradient.

The methods of measuring the intensity of selection which are mentioned above all treat selection as if it acted on one phenotype at a time, and therefore do not take account of the correlation between measurable characters. Such correlations can introduce a high level of complexity into the measurement of selection (Lande and Arnold 1983). Direct selection on one phenotype may result in indirect selection on another phenotype because it is correlated to the first. Furthermore, these methods of measuring selection are more descriptive than predictive (Lande and Arnold 1983), and are therefore not easily applied to predicting the change in phenotypes as a result of selection. As Falconer suggested (1981 chapter 20) the change in the metric character as a result of selection is of great interest, but cannot be assessed without taking into account the correlation between phenotypic characters. Kelly (1992) suggests that in considering traits of a plant's life history, selection may act directly at some stages of the plant's life history, and indirectly at others through selection on correlated traits. It is therefore important to take into account the selection on a trait through the plant's life-time. For example, time of emergence of *Chamaecrista fasciculata* is acted upon early in the plant's life history, but also indirectly through correlated traits later on in the life history of the plant (Kelly 1992).

Lande and Arnold (1983) propose a general solution of the regression method of Robertson (1966) and Price (1970) which utilises multivariate linear and quadratic regression techniques in order to take account of correlations between phenotypic characters.

According to the general multivariate regression method, directional selection is described in terms of the coefficients of the linear terms of the multiple regression analysis. The directional selection gradient of a character is then the change in fitness attributed to a unit change in the character. Stabilising selection is described in terms of the coefficients of the quadratic terms of the multiple regression analysis. In the general solution of Lande and Arnold (1983), the quadratic terms are derived from the square of each character minus its variance, and also a set of quadratic terms derived from the products of each combination of two characters minus the covariance of the two characters. The stabilising selection gradient is then the curvature described by the partial regression coefficients of these quadratic terms.

The object of the method is to generate a vector of partial regression coefficients: the linear terms then represent the directional selection gradient, and the quadratic terms the stabilising selection gradients. The general form of the quadratic regression of relative fitness on the characters is given in Lande and Arnold (1993) equation 16:

$$\Omega - 1 = \sum_{j=1}^{n} \beta_i z_j + \sum_{k=1}^{n} \sum_{l=k}^{n} (1 - \tfrac{1}{2}\delta_{kl}) \gamma_{kl} . [z_k z_l - P_{kl}] + \varepsilon \qquad 5$$

Here, the character values are given as the deviations from the mean character value. The notation is as follows:

1	Ω	represents relative fitness.
2	z_j	the deviation from the mean of jth character
3	β_j	the directional selection gradient on jth character
4	δ_{kl}	= 1 if k =l, and = 0 otherwise
5	τ_{kl}	the stabilising selection gradient on quadratic term
6	P_{kl}	the covariance (or variance if k = 1) between the kth and lth characters.

This equation requires the distribution of phenotypic characters to be multivariate normal. Unless this assumption is met, as Mitchell-Olds and Shaw (1987) discuss, the estimate of the coefficients of regression, and the tests of significance are unreliable. It is often the case that by transforming characters normality can be approximated, and Lande and Arnold (1983), in their own worked examples, do use log-transformed data.

Another difficulty with this method of measuring selection is that phenotypic characters which are not included in the estimate of the selection gradients may be exerting hidden effects on relative fitness, especially if they are correlated with those that have been included, and that therefore there will be a bias in the estimates of the selection gradients (Mitchell-Olds and Shaw, 1987). Careful choice of characters, based on an understanding of their significance in the life history of the organism may go some way to reduce such bias.

Mitchell-Olds and Shaw (1987) also point out that characters which are highly correlated cannot strictly be treated in a multivariate manner. The principle behind multiple regression is that the set of partial regression coefficients describes the change in the dependent variable due to a unit change in one independent variable, assuming all other independent variables are constant. If independent variables are correlated, one variable cannot change while holding the others constant. Consequently, the nature of the selection pressure revealed by multiple regression is conditional on the actual correlation between characters. A different set of correlation coefficients between the characters would produce a different set of selection gradients. Mitchell-Olds and Shaw (1987) support Lande and Arnold (1983) in their advice that with highly correlated characters, it may be advisable to reduce the number of characters used in the analysis.

The success of any method of measuring selection depends on how well relative fitness can be measured. Relative total lifetime fitness is extremely difficult to measure, and is unlikely to be practicable in most cases. Lande and Arnold (1983), however, suggest that the consideration of major components of fitness, which are more easily measured, can give important insights into the forces of selection. The method they propose can be applied equally to components of relative fitness, such as lifespan, fecundity, mating success, and mortality, as well as to relative total lifetime fitness.

The method of measuring selection of correlated characters by multivariate regression has been deployed extensively since Lande and Arnold first developed it. For example, Conner et al (1996a, 1996b), examined the selection gradients on a number of floral traits of the wild

radish (*Raphanus raphanistrum*), by regressing these traits against lifetime fecundity, and against lifetime male fitness. They identified strong selection for increased flower production, increased ovule number (in two out of three years), and flower size (in one year), with respect to fecundity, but found no significant selection with respect to male fitness.

Miller, Winn and Schemske (1994) discovered that early seedling emergence of *Prunella vulgaris* had a selection advantage over later emerging plants, especially at high densities.

Gomez (1993) studied the selection of flowering synchrony, flowering duration and flowering peak date using the multivariate regression method. In this case no consistent pattern of direct or stabilising selection was identified, although directional selection for flowering peak date was significant in one year, and stabilising selection for flowering peak date was significant in a different year. Similarly, Dominquey and Dirzo (1995) discovered that directional selection associated with variation in flowering initiation day of *Erythroxylum havanense* was negative in one year, but positive in the next.

Selection as a result of interspecific interactions has also been investigated using the multiple regression method. An insect/plant interaction has been investigated by Simms and Rauscher (1989a, 1989b) who analysed the selection on the annual *Ipomoea purpurea*, attributable to insect herbivory, to which there is variable resistance between plants. Selection pressure on *Eurosta solidaginis*, an insect which oviposits and induces galls in the stems of golden rod, is exerted by its natural enemies (Weiss, Abrahamson and Andersen 1992; Weiss and

Kaplinski 1994). Selection was found to be in favour of large galls with respect to parasitoid attack, but in favour of small galls with respect to predation by birds.

In the work that follows, the selection pressure on phenotypic characters of *Centaurea nigra* as a result of its interaction with *Urophora jaceana* is investigated. The plant characters considered are the following:

1 **Day of budding** defined as the day on which the leading bud of a plant first emerges more than 50% from its enveloping leaves.
2 **Size of bud on day of budding** defined as the diameter of the leading bud looking down from above.
3 **Day of flowering** defined as the day on which the first purple florets break out of the bud.
4 **Number of flowers on the plant.**

For day of budding and for day of flowering, the days are counted from 1st June as day one.

The component of fitness which is investigated in relation to characters of *Centaurea nigra* is annual fecundity, which as we have noted previously (Chapter 9, Section 1) is significantly reduced by galling.

12.1.2 Method

In late May 1993 a random sample of 30 *Centaurea nigra* plants in a 20 m^2 area of the field study site were tagged using strands of wool. The site was visited daily and each tagged plant was examined. As the first

bud emerged from its enveloping leaves, it was marked with a sticky paper label, with a reference number written on it, and the diameter of the bud (see Chapter 2: General methods) and the day of budding were noted.

As the marked buds began to break into flower, the day on which purple florets first became visible was noted for each marked leading bud. The number of flowerbuds on each plant was counted when all buds were in flower.

Between five and seven flowerheads from each plant were then harvested when seeds were about to ripen, at the end of August and beginning of September. These flowerheads were dissected, and the number of galls cells in each flowerhead, the number of eaten seeds, and the number of whole seeds were recorded.

12.1.3 Results

The total number of seeds, whole plus eaten seeds, was calculated for each flowerhead, and the mean number of seeds per flowerhead per plant was determined (Table 64). An estimate of the mean number of seeds lost per flowerhead by galling was made by multiplying the mean number of galls by the seed loss per gall, 7.3, as calculated in Chapter 9, Section 1. Added to the total seed production, an estimate of mean seed production in the absence of galls can be arrived at (Table 64).

The normality of the four characters was found to be improved by transformation. With respect to day of budding, size of bud and day of flowering, a better approximation to the normal distribution was obtained by using the logarithm of the characters. With respect to the number of of flowers per plant, the square root transformation was used.

Table 64: The day of budding, size of bud, day of flowering and number of flowerheads of 30 *Centaurea nigra* plants, with the mean number of seeds per flowerhead (and standard error), the mean number of gall cells per flowerhead (and standard error) and the estimated number of seeds per flowerhead if galls were not present. The estimate of the mean number of seeds lost per flowerhead by galling was made by multiplying the mean number of galls by the seed loss per gall, 7.3, as calculated in Chapter 3.1. This quantity was added to the total seed production to give the estimate of mean seed production in the absence of galls.

Day of budding	Size of bud	Day of flowering	Number of flowers	Seeds per flowerh'd	Seeds per flowerh'd (estimate)	Galls per flowerh'd
15	4.6	48	12	10.3 (7.4)	39.7	4.0 (1.3)
18	4.1	48	9	16.2 (7.0)	47.9	4.3 (1.9)
19	3.4	53	7	23.2 (5.4)	56.9	4.6 (1.3)
19	3.8	56	9	9.0 (3.0)	61.5	7.2 (0.9)
21	3.9	54	93	11.9 (7.8)	52.7	5.6 (1.7)
22	3.7	54	22	37.2 (1.8)	51.9	2.0 (0.2)

Table 64 contd....

Day of budding	Size of bud	Day of flowering	Number of flowers	Seeds per flowerh'd	Seeds per flowerh'd (estimate)	Galls per flowerh'd
22	5.0	51	34	20.7 (0.8)	59.8	5.3 (0.2)
22	3.7	55	24	27.7 (1.6)	69.2	5.7 (0.1)
24	4.0	59	42	26.2 (4.2)	44.5	2.5 (1.2)
25	4.0	60	32	33.7 (6.6)	63.0	4.0 (0.4)
26	3.7	61	7	21.2 (9.1)	24.8	0.5 (0.4)
27	4.6	61	15	32.2 (6.3)	49.3	2.3 (1.0)
28	4.2	60	15	41.8 (3.4)	54.1	1.7 (0.7)
28	3.8	62	67	37.8 (3.6)	43.9	0.8 (0.5)
28	3.7	60	8	27.7 (6.8)	43.6	2.8 (0.8)
28	4.3	62	28	19.7 (6.0)	28.2	1.2 (0.6)
29	3.8	63	7	35.0 (7.0)	42.3	1.0 (0.5)
29	3.7	63	17	41.8 (8.2)	61.4	2.7 (0.9)
29	3.6	64	16	39.6 (2.9)	57.2	2.4 (1.2)
29	4.1	62	22	44.7 (5.2)	54.4	1.3 (0.5)
29	3.9	62	38	51.0 (2.6)	55.4	0.6 (0.5)
30	3.6	66	12	46.0 (3.3)	48.4	0.3 (0.3)
30	4.3	65	5	33.0 (11.0)	37.9	0.7 (0.5)
30	4.2	66	46	46.5 (6.4)	64.8	2.5 (0.8)
31	3.7	66	24	53.8 (4.2)	56.3	0.3 (0.3)
33	3.8	71	33	47.8 (4.9)	52.7	0.7 (0.4)
34	3.8	70	12	52.3 (2.8)	57.2	0.7 (0.3)
34	3.6	71	12	27.8 (6.5)	45.4	2.4 (0.9)
34	3.8	69	6	37.5 (5.4)	39.3	0.3 (0.2)
36	3.7	69	8	34.4 (6.8)	53.5	2.6 (1.1)

Table 65: Correlations of the four characters of the 30 *Centaurea nigra* plants, with each other.

ns not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$.

	Day of budding	Size of bud	Day of flowering	Number of flowers
Day of budding	1.00	-0.28 ns	0.96 **	-0.13 ns
Size of bud		1.00	-0.36 *	0.13 ns
Day of flowering			1.00	-0.13 ns
Number of flowers				1.00

Table 66:

Quadratic terms in the multivariate regression analysis.

Character:		Day of budding b	Size of bud s	Number of flowers n
Day of budding:	b	b*b	b*s	b*n
Size of bud:	s		s*s	s*n
Number of flowers:	n			n*n

The correlations between day of budding and day of flowering (0.96, sig at $p < 0.005$ and between day of flowering and size of bud (-0.36, sig at $p < 0.05$) are significant but that between day of budding and size is not (Table 65). The correlations of number of flowers per plant with the other three characters were also non-significant (Table 65). Since the correlation between the day of budding and day of flowering is so high, and both characters refer to developmental traits of the flowerhead, it was decided to omit day of flowering from the analysis.

The deviations from the mean of the three remaining transformed characters were then calculated, and these were used in the multivariate regression analysis which follows. In addition, the six quadratic terms (Table 66) of these deviations and the variance or covariance, as appropriate, of each term was derived, from which the quadratic variables of Equation 1 could be calculated by subtracting the variance or covariance from the deviation from the mean of the equivalent quadratic term.

The deviations from the mean of the mean seed production per flowerhead, and of the estimate of mean seed production per flowerhead without galls, were also calculated.

A multiple analysis of covariance was performed (Crawley 1993, Chapter 12.5.4), using the GLIM programme with the normal error structure and identity link, in order to determine the partial regression coefficients of each character and each quadratic term on relative fitness (Table 69). The mean number of seeds per flowerhead of the gall-infested plants, and the estimated number of seeds per flowerhead if plants were not infested (Table 64), gave one factor with two levels, gall infestation and

non-gall infestation, with thirty replicates per level. The analysis of covariance enabled the difference in coefficients between the two factors to be tested.

The main effect of gall infestation was found to be insignificant, but when the nine interaction terms were fitted, there was a significant reduction in deviance (Table 66) at $p < 0.01$, which indicates a significant difference in slope between the two levels of the factor.

By removing each of the independent interaction terms, one at a time, from the full multiple regression model in turn, the increase in deviance which resulted was used to test for significance of each term. The F ratio of the interaction term is given by the increase in deviance divided by the scale parameter (Crawley 1993, Section 12.5.4) (Table 67).

Table 67: Analysis of covariance of the deviations from the mean of the mean number of seeds per flowerhead with and without gall infestation assuming different slopes between the factor levels (gall infestation and non gall infestation). Normal error structure and identity link. ** significant at $p < 0.01$; * significant at $p < 0.05$; ns not significant.

	Sum of squares	Degrees of freedom	Mean sum of squares	F ratio
Among factors	3.58	18	0.199	4.33 **
Within factors	1.88	41	0.046	
Total	5.46	59		

Table 68: Coefficients of each pair of interaction terms in the analysis of covariance. Standard errors of coefficients are given in brackets. The F-ratio is obtained in the GLIM programme by removing each interaction term in turn from the full model, and dividing the increase in deviance by the scale parameter. Normal error structure and identity link. ** significant at p < 0.01; * significant at p < 0.05; ns not significant.

Intercept	0.093 (0.041)			
Parameter	Coefficient		s.e.	F-ratio
	Infested plants	Non infested plants		
Day of budding	3.22	0	(0.67)	22.86 **
Size of bud	-2.32	-1.27	(1.69)	2.43 ns
Number of flowers	0.096	0.098	(0.033)	14.77 **
Day of budding x day of budding	1.37	9.04	(6.43)	2.00 ns
Size of bud x size of bud	25.18	15.90	(31.52)	0.89 ns
Number of flowers x number of flowers	-0.030	-0.031	(0.012)	9.72 **

Table 68 contd....

Day of budding x size of bud	9.27	25.73	(16.47)	2.69 ns
Day of budding x number of flowers	0.34	-0.41	(0.37)	2.39 ns
Size of bud x number of flowers	-1.85	0.16	(1.13)	2.74 ns

Scale parameter: 0.0458

Table 69: Significant Directional and Stabilising Selection Gradients acting on flowerhead characters of gall infested and non gall infested plants. All significant at $p < 0.01$.

Character	Directional Selection		Stabilising Selection	
	Gall infested	Non gall infested	Gall infested	Non gall infested
Day of budding (log transformed)	3.22 (0.67)	-	-	-
Number of flowers (square root transformed)	0.096 (0.033)	0.098 (0.033)	0.015 (0.006)	0.015 (0.006)

The significant terms in the analysis of covariance are the interaction of the gall infestation factor with day of budding, with the number of flowers, and with the number of flowers x number of flowers, all significant at $p < 0.01$ (Table 68). When the coefficient for day of budding on non-infested plants was aliased (set at zero) it was found not to increase significantly the deviance of the model. The coefficients for day of flowering, and day of flowering x day of flowering were all found to be significant.

The significant selection gradients, as calculated from Table 68 and Equation 1 are shown in Table 69.

12.1.4 Discussion

Directional selection appears to act on day of budding, log transformed, with a gradient of 3.22, when the plants are gall-infested. When plants are not infested, there is no directional selection on day of budding. Directional and stabilising selection acts on the number of flowers, square root transformed, with no significant difference in intensity between plants which are infested and those which are not. The gradients are 0.096 and 0.015 respectively.

Interpreting these selection gradients, gall infestation appears to exert a selection pressure on the plant. It is the case that when gall infestation occurs, selection favours those plants which bud later, and which bear more flowers. Concerning budding time, it was found that this character is heritable, $h^2 = 0.36$ approximately (Section 2).

However, there is a compensating factor which may affect the situation. Since the directional selection gradient on number of flowers is positive, larger plants, bearing more flowers, are favoured by selection. Put another way, gall infestation of larger plants is at a lower density than on smaller plants, and therefore produce more seed per flowerhead. Thus a large, early budding plant may be as competitive as a small late budding plant. However, the curvature, measured by the stabilising selection gradient, of the relationship between number of flowers and relative fitness without galls, means that the increase of fecundity per flowerhead falls off as plants become larger, until a maximum fecundity is reached, probably because there is a physical limit to the number of seeds per flowerhead.

When plants are not gall-infested, there is no selection, directional or stabilising, on day of budding. However, there is still directional and stabilising selection on the number of flowers.

Caution must be observed in evaluating these results in terms of selection from year to year, in view of the findings of Gomez (1993) and Dominquez and Dirzo (1995), mentioned above, who found that selection on characters varied between years.

In conclusion, gall infestation affects the gene frequency of the population, whereas plant population recruitment is not affected (Chapter 9). So this is a case where individual level processes affect some (gene frequency) but not other (population recruitment) population parameters.

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Section 2: To investigate the heritability of phenotypic characters of *Centaurea nigra*

The aim of this section is to test whether any of the flowerhead phenotypic characters of *Centaurea nigra* are genetically based, and therefore heritable. The purpose of doing this test is to determine whether the selection pressure on the phenotypes as a result of galling has implications for the evolution of the plant-insect interaction.

Heritability tests were applied to four characters:

1 Day of budding

2 Size on day of budding

3 Day of flowering

4 Height of the leading stalk on the day of budding.

The experimental design followed the example given by Lawrence (1984) for natural progenies. Despite the limitations of this design for an accurate determination of heritability, it was considered adequate to answer the fundamental question: which flowerhead characters are heritable?

12.2.1 Introduction

Many quantitative differences between individuals in a population can be attributed to a combination of the effects of the environment and the effects of genotypic variation. The date of flowering of *Centaurea nigra* displays a wide variation both within and between populations. From

year to year, there are differences in date of flowering of the same population (Lack 1982), suggesting that there is an environmental component of variation. The differences in date of flowering between a number of populations was maintained in cultivation (Lack 1982), suggesting that the variation also has a genetic component. The purpose of performing heritability tests is to separate out environmental and genetic components of variation in measurable traits.

The broad sense heritability (Bradshaw 1984) of a character (h^2_B) indicates the degree to which a metrical character is genetically determined, h^2_B being the proportion of the variation in a population which is explained genetically. The narrow sense heritability (Bradshaw 1984) (h^2_N) is the degree to which a character is transmitted to offspring, and is derived from the additive genetic variance.

Heritabilities are calculated for specific populations (Lawrence 1984), because each population carries a gene pool (Crawford 1984) which is characteristic of that population. Heritability is therefore a characteristic of the population (Falconer 1981). Differences between populations are illustrated by Wolff and Van Delden (1986) for *Plantago lanceolata* in four populations, in which some characters are found to be heritable in some populations but not in others, for example flowering date.

There are two requirements (Lawrence 1984) for the carrying out of heritability tests:

1 The recognition of groups of related organisms;

2 The separation of the effects of environment from the effects of genotype on phenotypic variation.

Groups of relatives, or families, suitable for heritability tests are parent - offspring, full sibs, and maternal and paternal half sibs. All these types of families require experimental work in order to be able to recognise the families, because in wild populations it is rarely possible to identify sibs or parents and offspring (Lawrence 1984).

Separation of the effects of environment from effects of genotype variation can only be done in a controlled situation. Lawrence (1984) suggests that the random positioning of individuals, and replication, enable a statistical separation to be achieved.

The choice of experimental design depends upon the balance between theoretical and practical considerations. The ease of controlled cross-pollination, the number of individual plants required within each family group to give adequate statistical accuracy, the time required for the plants to reach maturity, and the ease of measuring the relevant metrical character, all need to be taken into account, and balanced against the aims of the experiment. Mitchell-Olds and Rutledge (1986) suggest that if accurate heritabilities are to be established, controlled pollinations are necessary.

In this instance, the difficulties of controlling cross pollinations of large numbers of plants flowering at different times, and the time for plants to reach maturity (at least two years) were the main considerations in judging the practicality of the design used. In view of the purpose of the experiment in the overall plan of the thesis, estimating heritabilities from natural progenies (Lawrence 1984, Vrieling 1990) was chosen as the most convenient design, while fully recognising the design's limitations.

First, selfing in *Centaurea nigra* is minimal (Marsden Jones and Turrill 1954). If *Centaurea nigra* is predominantly dioecious, it can be assumed that, with natural progenies drawn from one flowerhead of a plant, we are dealing with a mixture of full and half sibs. The heritability will then lie between an upper and a lower estimate, calculated as four times and twice the intraclass correlation coefficient.

Second, the families of sibs which are recognised will potentially be a mixture of selfs, full sibs and maternal half sibs, depending on the mechanisms of pollination involved. In this instance, an upper and a lower limit of heritability will be estimated (Lawrence 1984).

Third, if pollination takes place over limited distances, and near neighbours in the plant population are likely to be related, then the assorted sibs may be the progeny of inbreeding, which may seriously bias estimates of genetic variance (Mitchell-Olds and Rutledge 1986).

The fourth limitation is that dominance may be a significant factor which influences phenotypic variation, which is impossible to isolate and quantify by this method (Falconer 1981, Wolff and Van Delden 1986).

The fifth limitation is due to maternal effects on maternal half sibs, which are the result of differences of the maternal environment provided by individual mother plants in the population (Mitchell-Olds and Rutledge 1986, Antonovics and Schmitt 1986). In some cases, the maternal effects may outweigh any effects of genetic variance, for example, seed weight in variation in wild radish (Mazer 1987), whereas in other instances these effects are not significant, as in Pa-concentration in the shoots of *Senecio jacobaea* (Vrieling 1990).

The theoretical limitations caused by dominance and maternal effects are illustrated by the following equation:

$$t = \frac{1/2\ V_A + 1/4\ V_D + V_M}{V_P} \qquad \text{(Falconer 1981)}$$

where V_A is the additive genotypic variance

V_D is the dominant genotypic variance

V_P is the phenotypic variance

V_M is the variance due to maternal effects

and t is the intraclass correlation coefficient.

The equation above reveals that, at best, a natural progeny design will give only the broad sense heritability, because the effect of dominance is unknown. Furthermore, maternal effects will add bias to the estimate, because it is not possible to separate these effects out.

To summarise, heritabilities estimated from natural progenies will be broad sense heritabilities which will include the genetic effects of inbreeding and dominance. Maternal effects will also bias the estimates. Because the sib families are a mixture of full and half sibs, the heritability will lie between an upper and lower limit, given by:

$$h^2_B/2 \geq t \geq h^2_B/4 \qquad \text{(Lawrence 1984).}$$

12.2.2 Method

Seed from one ripe seedhead per *Centaurea nigra* plant was harvested from 15 individual plants randomly selected from the wild population at the field study site in Warmington, in September 1992. It is assumed that each family of harvested seed includes a mixture of full and maternal half sibs. The balance is likely to be in favour of full sibs.

The fifteen families of seed were sown separately in seed trays with multi purpose coir compost (B & Q) in February 1993 and germinated at room temperature (circa 16° C.) in light. When seedlings had developed to the stage of having approximately four proper leaves, twenty seedlings chosen at random in each family were transplanted into plastic Plant Pak pots of uniform size using general purpose coir compost.

The plants were allowed to grow on out of doors throughout the summer and autumn of 1993, ensuring regular watering. The plants were over-wintered out of doors, and transplanted into larger plastic Plant Pak pots using coir compost in late April 1994. By this stage, most plants had developed a healthy rosette of leaves, and some were beginning to develop flower stems.

Due to some mortality over the winter, families ranged in size from seventeen to twenty plants. From each family, 14 plants were chosen at random using a deck of cards. The 210 plants were arranged in a randomised block, situated in the garden, free of insect and mollusc herbivores, in a situation where conditions were as uniform as possible. Care was taken to water well in dry periods.

As the plants developed, they were examined daily in the early evening, and for each plant, the following measurements were recorded:

1 Date of budding: the day on which the first bud begins to show through enveloping leaves.

2 Diameter of first flowerhead on day of budding.

3 Height of budding stalk on day of budding.

4 Date of flowering: purple florets beginning to show through bracts on first flowerhead.

The dates of budding and of flowering were recorded in "June Days", and the diameters of flowerheads in millimetres, using external callipers and steel rule, calibrated to 0.5 mm, to the nearest 0.1mm.

12.2.3 Statistical Analysis

Heritabilities (h^2_B) of the five phenotypic characters were calculated using single classification analysis of variance (Sokal and Rohlf 1981, chapters 8 and 9). The general form of the anova table is shown in Table 70.

Table 70: General form of anova table to calculate heritability.

Source of variation	df	Mean Squares		Expected MS
Between Families	n-1	$MS_{(B)}$	=	$s^2_W + rs^2_B$
Within Families	n(r-1)	$MS_{(W)}$	=	s^2_W
Total	nr-1			

where r = number of sibs in each family

n = number of families

and $$s^2_B = \frac{MS_{(B)} - MS_{(W)}}{n} \qquad 1$$

The broad sense heritability, h^2_B is given by (Falconer 1984, chapter 10; Lawrence 1984; Vrieling 1990; Wolff and van Delden 1987):

$$h^2 = \frac{2s^2_B}{s^2_B + s^2_W} \qquad 2$$

and the 95% confidence limits by

$$\frac{2}{\left(\frac{1 + n*F_\alpha}{R - F_\alpha}\right)} < h^2 < \frac{2}{\left(\frac{1 + n*F_{1-\alpha}}{R - F_{1-\alpha}}\right)} \qquad 3$$

where R is the F_s value of the anova and F_α is the critical value of the relevant F distribution for α = 0.025. The data were analysed using the GLIM programme.

12.2.4 Results

The means and standard deviations of the four characters (day of budding, size on day of budding, day of flowering and height of stalk) for the 15 full sib families of 14 wild progenies are shown in Table 71.

Table 71: Means and standard deviations (in brackets) of four characters of the fifteen natural sib families. Number of progenies in each family is 14.

Day of budding	Size of bud	Day of flowering	Height of stalk
23.93 (8.08)	4.31 (0.381)	48.43 (11.26)	34.50 (8.72)
25.21 (4.34)	4.49 (0.657)	57.00 (6.94)	41.07 (9.34)
20.07 (10.26)	4.75 (0.519)	49.64 (6.38)	38.07 (7.62)
22.36 (4.34)	4.50 (0.227)	54.93 (4.07)	36.79 (7.19)
23.57 (4.70)	4.37 (0.691)	56.71 (5.55)	32.93 (5.40)
22.07 (3.75)	4.14 (0.568)	51.64 (4.22)	44.43 (10.29)
21.29 (5.01)	4.56 (0.457)	52.71 (4.92)	39.14 (6.72)
22.50 (4.94)	4.54 (0.715)	52.36 (5.69)	45.86 (6.47)
21.36 (4.05)	4.42 (0.430)	52.14 (4.99)	33.14 (7.09)
21.14 (4.93)	4.46 (0.499)	51.50 (5.52)	36.64 (9.15)
22.14 (2.71)	4.49 (0.353)	52.79 (3.31)	36.36 (10.05)
22.57 (6.06)	4.59 (0.582)	53.79 (6.41)	38.50 (7.62)
23.50 (4.18)	4.48 (0.518)	52.14 (4.93)	28.93 (7.94)
26.86 (7.74)	4.15 (0.403)	56.43 (3.57)	30.93 (6.96)
36.36 (13.77)	4.05 (0.445)	69.79 (16.06)	28.57 (8.51)

Table 72: Analysis of variance tables for the estimation of broad sense heritability of four plant characters.

In the Analysis of Variance, the data for day of budding, size of bud and day of flowering were transformed into natural logarithms in order to approximate better to normal distribution. The data for height of stalk was analysed untransformed.

Day of budding (transformed to logarithms)

	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	s^2_B	h^2_B
Among families	3.97	14	0.283	4.09	0.015	0.36
Within families	13.50	195	0.069			
Total	17.47	209				

Size on day of budding (transformed to logarithms)

	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	s^2_B	h^2_B
Among families	0.297	14	0.021	1.610	0.001	0.08
Within families	2.573	195	0.013			
Total	2.87	209				

Day of flowering (transformed to logarithms)

	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	s^2_B	h^2_B
Among families	1.16	14	0.083	5.697	0.005	0.50
Within families	2.82	195	0.015			
Total	3.98	209				

Height of stalk (untransformed)

	Sum of squares	Degrees of freedom	Mean sum of squares	Fs	s^2_B	h^2_B
Among families	5096	14	364	5.618	21.37	0.50
Within families	12634	195	65			
Total	17730	209				

There are a significant differences between the families of sibs, in the day of budding, the day of flowering, and in height of stalk, at $p < 0.01$. However, there is no difference in the size of bud between sib families (Table 72).

From the analysis of variance tables, the broad sense heritability, h^2_B, of each character, was calculated, with the 95% confidence limits, using the formulae 1, 2 and 3 above (Table 73).

Table 73: Heritabilities and 95% confidence limits of each character.

	Heritability h^2_B	95% Confidence limits of h^2_B	
Day of Budding	0.362	0.162 to 0.792	
Size of bud	0.084	-0.017 to 0.353	not sig.
Day of Flowering	0.502	0.262 to 0.969	
Height of Stalk	0.496	0.257 to 0.962	

The heritabilities of day of budding, day of flowering and height of stalk are significantly greater than zero, whereas size of bud does not appear to be heritable (Table 73).

12.2.5 Discussion

Maternal effects may have seriously biased these results, and without further investigation it is impossible to conclude whether the heritabilities shown in Table 73 are significant. However, the results are not inconsistent with day of budding and of flowering, and height of stalk being heritable in the broad sense.

If maternal effects are ignored (Weiner et al 1997), day of budding and day of flowering, which are highly correlated in any case, have estimated lower heritabilities of 0.362 and 0.502 respectively. This means that approximately 36% of the variation of day of budding and 50% of the variation in day of flowering in the population can be explained genetically. Stalk height at time of budding is also heritable, with h^2_B equal to 0.496. The size of buds at budding appears not to be heritable.

Compared to other plants, *Centaurea nigra* shows a similar pattern of heritability in some of these characters. For example, flowering date of *Plantago lanceolata*, as measured by the number of days between germination and appearance of first stamens, was found to be heritable in two out of four populations (Wolff and Van Delden 1986). Flowering date and height were both found to be heritable in wild radish, *Raphanus raphanistrum* (Mazer 1987). In this case flowering early was associated with a fitness advantage.

The overall conclusion of this chapter is that the directional selection acting on the day of budding as a result of gall infestation has a genetic component.

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Part 4:
Conclusion

Chapter 13: From Individual Behaviour to Population Level Phenomena.

13.1 Timing of oviposition

The interaction between the gall fly, *Urophora jaceana* and its host plant, *Centaurea nigra* focuses on one key point, that there is a narrow range of opportunity for oviposition in flowerbuds. In Chapter 5 it was found that the preferred stage of bud development for oviposition by the fly is when the bud emerges from its enveloping leaves. Typically, the size of bud at this stage is between 3.5mm and 5.5mm, but size is less important than stage of development as a cue for oviposition. This conclusion is corroborated by work on other tephritid flies, which appear to use a variety of different cues for oviposition in flowerheads of host plants (Straw 1989a, 1989b). Timing is therefore crucial in the search for suitable oviposition sites and may play a part in population regulation of *Urophora jaceana*.

13.2 Regulation of Gall Fly populations

Regulation of populations may be due to density-dependent predation by natural enemies, or the result of density-dependent competition for limited resources (Begon and Mortimer 1986, Chapter 7). Life table analysis of populations of *Urophora jaceana* (Varley 1947, Dempster et al 1995a) has produced no clear evidence of density dependent mortality due to natural enemies. Varley (1947) based his conclusions on just two years' data, and Dempster et al (1995a), comparing data from a number

of patches in the same season, found no statistically significant density-dependent relationships of *Urophora*, nor three other species of tephritid flies, with any of their parasitoids. In this thesis, four years of data were analysed (Chapter 10) with similar conclusions. Winter disappearance, probably due mainly to predation by small rodents, is the one factor which is approaching significance (Chapter 10), which corroborates the finding of Dempster et al (1995a) that density-dependence is more likely to be operating in the full grown larvae stage through to adult stage of the *Urophora* life cycle.

In view of the inconclusive evidence concerning density-dependent regulation by natural enemies, Dempster et al (1995a) suggest that competition for resources, in other words oviposition sites, in the adult stage is more likely to be the regulating factor at high densities. In situations where there are high densities of gall flies and limited numbers of oviposition sites, Dempster et al (1995a) found evidence of density-dependent competition between flies, leading to interference and to emigration from patches (Dempster et al 1995b).

The variation of budding times within and between *Centaurea nigra* populations, being highly correlated with flowering times, is quite large (Lack 1976, 1982, Elkington and Middlefell 1972, Turrill and Marsden-Jones 1954). Although *Urophora jaceana*, and indeed other tephritid flies which oviposit in flowerheads, appear not to utilise all available resources (Straw 1991), the distribution of resource availability within a season, as shown in Chapter 10, suggests that early in the season oviposition sites are limited and that, as the season progresses, sites become more plentiful as more plants come into bud. The density of gall flies emerging also varies with time. It is in the nature of the

interaction that sufficient synchrony is needed between these two distributions for a sufficient number of buds to be available for oviposition, although the life expectancy of flies in the field will determine how finely tuned the synchrony has to be. Although there is insufficient evidence to assess life expectancy of *Urophora jaceana*, it is likely to be much shorter than the time period when suitable buds are available, and indeed may be as short as six or seven days on average (Varley 1947). There may be situations in which most buds are available for oviposition at a time when few gall flies have emerged, or at a time when many gall flies have died. It is of course possible that this is one reason for local extinctions of gall flies in isolated patches (Dempster et al 1995a).

13.3 Selection on day of budding of the plant as a result of gall infestation

The effect of gall infestation on the plant population is to reduce seed production significantly, although seedling recruitment and survival were not affected (Chapter 9). The seed production of individual plants was also greatly reduced, but plant resources were not diverted from elsewhere in the plant into galled flowerheads (Chapter 9), and galls appear not to act as nutrient sinks (Myers and Harris 1980, Harris 1980). The effects of gall infestation are restricted to the reduction of seed production in individual flowerheads which are galled. Selection on plants as a result of gall infestation will therefore be focused on fecundity as a component of fitness (Christiansen 1984, Kozlowski 1993).

The specificity of the timing of the insect-plant interaction, and the densities of buds suitable for oviposition and of gall fly emergence, combine to produce the within-season distribution of galling described in Chapter 11. This within-season distribution of gall infestation appears, therefore, to drive the directional selection on day of budding which was identified in Chapter 12. The early budding plants are more heavily galled than later ones, and because seed production is inversely dependent on the level of galling, the relative fecundity of early plants is less than later ones.

Day of budding may be heritable, although the sib analysis from natural progenies is inconclusive due to confounding maternal effects with dominance and additive genetic effects (Lawrence 1984, Falconer 1981). Natural selection, driven by the gall fly, may therefore be acting on the plant. However, annual variations of budding time, due to the phenotypic plasticity of the plant and varying weather conditions, and annual variations in eclosion times of gall flies, would result in the intensity, and even direction of selection, changing from year to year. Such variable selection is not uncommon in wild populations (Gomez 1993, Dominquey and Dirzo 1995, Kelly 1992, Weiss et al 1992, 1994)

Akimoto and Yamaguchi (1994) found that budding time of the host plant (*Ulmus davidiana*) exerts a selection pressure on the gall aphid *Tetraneara sp.* through the process of gall formation, and that *Tetraneura* populations on phenotypically distinct trees become genetically differentiated (Akimoto 1990, see also Dongen et al 1997). In Chapter 11 it was discovered that the proportion of *Urophora jaceana* larvae attacked by *Eurytoma curta* in early budding flowerheads was

less than in later budding flowerheads. Phenotypic selection may therefore also be acting on the timing of gall formation of *Urophora* larvae through the specificity of attack by this parasitoid.

13.4 The Dynamic State Variable Model

The interaction between *Urophora jaceana* and its host plant is highly specific, focusing on the timing of oviposition in host buds. Adult *Urophora* spend most of their time on the host plant (Varley 1947) and moving between host plants. An individual female makes choices concerning where and how long to search, whether to oviposit in a bud which has been encountered and how many eggs to oviposit (Chapter 7). The Dynamic State Variable Model (Mangel and Clark 1988) derived in Part 2 of this thesis attempts to quantify these decisions over the life time of individual flies. The key concept in the model is the optimisation of Lifetime Expected Fitness, through oviposition decisions about whether to super-oviposit and how many eggs to oviposit (Chapter 3).

The Dynamic Modelling Equation (Chapter 7) rests on the density-dependence of larval mortality up to gall formation (Chapter 6), and on the differential survival of larvae from gall formation to third instar in flowerheads with less than or more than seven gall cells (Chapter 6). It also incorporates a random search parameter, with the encounter rate calculated from direct observation of individual foraging flies in the field (Chapter 4), and the two state variables of egg load and the distribution between parasitised and unparasitised host density.

The model predictions are consistent with those of Mangel and Clark (1998) and Mangel (1987). Clutch size increases at lower host density, increases with higher egg loads and increases with the age of the insect (Chapter 7). Super-oviposition is more likely to occur at low densities of hosts, high densities of flies, high egg load, and with the age of the insect (Chapter 7). The prediction that super-oviposition was more likely to occur at high host encounter rates was shown to be consistent with experimental results (Chapter 7).

The overall predictions of the model are the result of density-dependent processes at work within clutches. Pre-gall formation, competition between larvae establishing gall inducing positions within the flowerhead may be the important factor (Varley 1947). Post-gall formation, preference of lepidoptera larvae for flowerheads with large gall complexes lead to differential survival of larvae between small and large gall complexes (Chapter 6).

Population regulation of *Urophora jaceana* can be understood partly in terms of the behavioural decisions of individual females, as predicted by the model. At low densities of hosts the clutch sizes oviposited will be higher than when host densities are much higher. The incidence of super-oviposition will also be greater at low densities of hosts. At high densities of eggs per flowerhead, typically when oviposition sites are limited, density-dependent competition between larvae pre-gall formation, and predation by lepidopteran larvae post-gall formation, will be important regulating factors.

The Dynamic Model was also used to predict population level effects using the method of Monte Carlo Simulation (Chapter 8). The key prediction here concerning population regulation is the convex curvature of the proportion of flowerbuds parasitised when plotted against fly density (Chapter 8). This curvature arises out of the indirect interference of individual females searching for oviposition sites. Each flowerbud encountered by one individual will reduce the probability of other flies encountering unparasitised flowerbuds. So the model predicts the sort of density dependent competition suggested by Dempster et al (1995a, 1995b) as an important regulating factor of gall fly populations. The search persistence of flies in patches may be related to the encounter rate with unparasitised hosts (Roitberg and Prokopy 1984), and may provide a dynamic for emigration decisions.

How far the population predictions of the Dynamic Model are borne out in the field could be the subject of extensive investigation, which would draw out some limitations of the model. At the population level, immigration and emigration in and out of patches (Dempster et al 1995b), and the continuously changing availability of oviposition sites could be incorporated into the model. In terms of individual decisions, some assumptions built into the dynamic modelling equation, such as random search and pro-ovigenity, may be found to bias the model. Parameters such as the clutch size fitness function, foraging life expectancy, and length of foraging day, need to be refined. The effects of weather on behaviour and mortality as a function of age could be investigated.

Any refinement and development of the model would need to balance its descriptive accuracy against the greater complexity resulting by introducing more variables into it (Gladstein et al 1991). Critical

assessment of the relative importance of different variables, through sensitivity analysis (Gladstein et al 1991), is crucial in curtailing the complexity of dynamic models, which makes them too unwieldy to be useful.

Individual-based models, despite limitations, are a promising way of gaining new insights into population processes. In the field of ornithology, an individual-based, game theory model is being developed for the distribution of oystercatchers (*Haematopus ostralegus*) feeding on mussels (*Mytilus edulis*) (Goss-Custard et al 1995a,b,c). There are three main advantages of this approach.

1 The difficulties of obtaining data over a sufficiently long time series concerning the population trends of the organism are avoided (Goss-Custard et al 1995a). The study of individual behaviour requires a shorter timescale, and the parameters derived can be incorporated into models which apply to a wider range of environmental conditions.

2 There is potential for comparisons between systems which are not easily made in species-specific long-term population studies (Goss-Custard et al 1995c). In particular, individual-based models will highlight the processes behind density dependence, such as interference (Goss-Custard et al 1995c).

3 It is possible to make predictions concerning future population trends arising out of significant changes in the environment. For example, Goss-Custard et al (1995b) predict what might happen to oystercatcher populations in response to habitat loss.

The simple, individual-based model for *Urophora jaceana* and its host plant *Centaurea nigra*, developed in this thesis, while having limitations, indicates some of the population consequences of individual behaviour.

1 Clutch size is dependent on the density of buds suitable for oviposition.

2 Super-oviposition is incorporated as an optimal strategy in certain circumstances.

3 Density-dependent processes within gall complexes in individual flowerheads may play a part in population regulation.

4 There is indirect interference between flies competing for oviposition sites, which is dependent on fly density.

5 The individual behaviour of *Urophora jaceana*, combined with the phenotypic distribution of the plant host, especially of day of budding, results in directional selection on the plant.

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Appendix: Computer programme of Dynamic Model

The computer programme is written in Locomotive Basic 2, on a 640K RAM personal computer. The relatively small amount of memory available for the programme meant that very large arrays had to be avoided, and compromises had to be made concerning the number of time periods and the initial egg load of flies. Using the LET command, which allows values to be redefined, and by running the programme in sections, difficulties over the size of memory were overcome. the programme presented here, for the purposes of simplicity, assumes unlimited memory.

Table 74 shows the arrays and input for the computer programme. The array f(75,70,300) represents the expected lifetime fitness function, with 75 time intervals, an initial eggload of 64, and a variation in clean and parasitised host numbers of 300. End Time is period 75. Arrays n(2) and h(2) define initial clean and parasitised host numbers. Array w(1,8) represents the solution to the clutch size survival equations (equations 4 and 7 above), and array v(1,8) represents clutch size survival plus expected lifetime fitness for each clutch size. It is redefined in the programme for each eggload, host distribution and time interval.

Arrays z(11) and m(11) are both random numbers, and q(11) defines the host encountered, r(11) the clutch size, and s(11) the type of host, in the MOnte Carlo part of the programme, up to a maximum of 12 flies (0 to 11). Array i(11) represents the eggloads of 12 flies, which are redefined after each time interval in simulation. Array l(11,8) is comparable to array v(1,8), but refers to actual fitness increments as

flies make choices. Array g(300) keeps track of the number of eggs laid in each host, and y(1) enables the mean and variance of eggs laid per host to be calculated.

Initial host number, a, and initial clean and parasitised host numbers. n(1) and n(2), are set in line 130. The number of flies is set by the variable o, which allows fly numbers of 3, 6, 9, and 12.

Table 74: Definition of arrays and input of initial host and fly numbers

For explanation see text

```
100   DIM f(75,70,300): DIM h(2): DIM n(2): DIM w(1,8): DIM v(1,8)
110   DIM z(11): DIM m(11): DIM q(11): DIM r(11): DIM s(11)
120   DIM i(11): DIM l(11,8): DIM g(300): DIM y(1)
130   INPUT "hosts"; a: INPUT n(1): input n(2)
140   LET h(1) = n(1): LET h(2) = n(2)
150   INPUT "flies"; o: FOR b = 0 to 11: LET i(b) = 70: NEXT b
```

In Table 75, lines 180 to 230 calculate the survival functions w(0,c) and w(1,c) for clutch sizes zero to eight. Line 240 calculates the Lifetime Expected Fitness for all values of eggload and host distribution at time interval 75, which is the penultimate time interval, assuming that the Lifetime Expected Fitness at the end time is zero. Lines 270 to 330,

cycling through all time intervals from 74 to one, eggloads from 1 to 64 and number of parasitised hosts from 0 to 200, calculate the Lifetime Expected Fitness, f(74-t,x,d). Line 320 is analogous to equation 8 above.

Table 75: Calculating Future Lifetime Expected Fitness

```
170   For t = 0 to 73: for d = 0 to 200: LET f(t,0,d) = 0: NEXT d: NEXT t
180   FOR x = 1 to 70: FOR d = 0 to a: FOR c = 1 to 8
190   IF x < c THEN LET w(0,c) = 0: IF x < c THEN LET w(1,c) = 0: IF x
      < c GOTO 220
200   LET w(0,c) = (-0.0396 + 1.02*c - 0.0204*c*c)*0.52
210   LET w(1,c) = ((-0.0396 + 1.02*(8+c)
                                  - 0.0204*(8+c)*(8+c)))*c/(8+c))*0.34
220   NEXT c
230   LET w(0,0) = 0: LET w(1,0) = 0
240   LET f(75,x,d) = (1-EXP(-0.004*a))*((h(1)-d)/a)*MAX(w(0,0),w(0,1),
      w(0,2),w(0,3),w(0,4),w(0,5),w(0,6),w(0,7),w(0,8)) + (1-EXP(-0.004*a))
      *(d/a)*MAX(w(1,0),w(1,1),w(1,2),w(1,3),w(1,4),w(1,5),w(1,6),w(1,7),w(1,-
      8))
250   NEXT d: NEXT x
270   FOR t = 0 to 73: FOR x = 1 to 70: FOR d = 0 to a-1
280   FOR c= 0 to 8: IF c < c THEN LET v(0,c) = 0: IF x < c THEN LET
      v(1,c) = 0: if x < c THEN GOTO 300
290 LET v(0,c) = w(0,c) + f(75-t,x-c,d+1): LET v(1,c) = w(1,c) +
      f(75-t,x-c,d)
300   NEXT c
```

```
310   LET f(74-t,x,a) = EXP(-0.004*a)*f(75-t,x,a) + (1-EXP(-0.004*a))
      *((h(1)-a)/a)*MAX(v(0,0),v(0,1),v(0,2),v(0,3),v(0,4),v(0,5),v(0,6),
      v(0,7),v(0,8)) + (1-EXP(-0.004*a))*(a/a)*MAX(v(1,0),v(1,1),
      v(1,2),v(1,3),v(1,4),v(1,5),v(1,6),v(1,7),v(1,8))
320   LET f(74-t,x,d) = EXP(-0.004*a)*f(75-t,x,d) + (1-EXP(-0.004*a))
      *((h(1)-d)/a)*MAX(v(0,0),v(0,1),v(0,2),v(0,3),v(0,4),v(0,5),v(0,6),
      v(0,7),v(0,8)) + (1-EXP(-0.004*a))*(d/a)*MAX(v(1,0),v(1,1),
      v(1,2),v(1,3),v(1,4),v(1,5),v(1,6),v(1,7),v(1,8))
330 NEXT d: NEXT x: NEXT t
```

In the Monte Carlo Simulation, the programme cycles through the time intervals from u = 0 to 74, the End Time, as indicated in line 370 of Table 76. A variable number of flies may be used in the simulation, up to 12. I chose to run the simulation with three, six, nine and twelve flies. In each time interval, each fly, b, is assigned a random number, z(b), which determines the type of encounter experienced by the fly in that time interval. Three types of encounter are allowed: no host, a clean host, or a parasitised host. The three types are modelled in lines 390+400, 410 and 420 respectively. If no host is encountered, the eggload and the host distribution remain the same, and the simulation proceeds to the next fly. If a clean host is encountered, the simulation proceeds to Table 4, line 440, in order to determine the size of clutch which the fly will lay in the host. If a parasitised host is encountered the simulation proceeds to Table 78, line 560.

Table 76: The Monte Carlo simulation: 1

```
370  FOR u = 1 TO 75: LPRINT u, n(1): FOR b = 0 to 3*o-1: LET z(b) =
     RND
380  IF i(b) < 1 THEN LET r(b) = 0: IF i(b) < 1 THEN GOTO 760
390  IF z(b) > (1-EXP(-0.004*a)) AND z(b) <= 1 THEN LET r(b) = 0
400  IF z(b) > (1-EXP(-0.004*a)) AND z(b) <= 1 THEN GOTO 760
410  IF z (B) >= 0 AND z(b) <= (1-EXP(-0.004*a))*(n(1)-n(2))/a
     THEN GOTO 440
430  IF z(b) >= (1-EXP(-0.004*a))*(n(1)-n(2))/a
     AND z(b) < (1-EXP(-0.004*a)) THEN GOTO 560
```

Table 77: The Monte Carlo simulation: 2

```
440  LET s(b) = 0
450  FOR c = 0 TO 8
460  IF i(b) < c THEN LET l(b,c) = 0: IF i(b) < c THEN GOTO 480
470  LET l(b,c) = w(0,c) + f(u+1,i(b)-c, n(2)+1)
480  NEXT c
490  FOR c = 0 to 8
500  IF l(b,c)    =    MAX(l(b,0),l(b,1),l(b,2),l(b,3),l(b,4),l(b,5),l(b,6),
                       l(b,7),l(b,8)) THEN LET r(b) = c
510  NEXT c
520  IF r(b) > 0 THEN LET n(1) = n(1)-1: IF r(b) > 0 THEN LET n(2) =
     n(2)+1
```

Table 77 contd....

```
530   IF r(b) > 0 THEN LET q(b) = n(2)
540   LET g(q(b)) = r(b)
550   GOTO 700
```

Table 77 shows how the programme determines the size of clutch laid by the fly in a clean host. Line 470 defines l(b,c), which is the fitness increment gained by laying a clutch of size c plus the future lifetime expected fitness. Line 500 simply determines the value of c which gives the maximum value of l(b,c). The value of c then gives the clutch size. Lines 520 to 540, then redefine the host distribution, and also assigns a reference number (q(b) to the newly parasitised host.

Table 78 shows how the programme determines the clutch size laid by the fly if it encounters a parasitised host. First the reference number of the particular host encountered is determined using a random number m(b), in line 560. The number of eggs in the host before the encounter is thus known. The procedure then follows that in Table 4, except that here, not laying an egg is a distinct possibility, equivalent to a clutch size of zero.

Line 680, in Table 79, revalues the number of eggs laid in the parasitised host with reference q(b). Lines 700, 710 and 720 indicate the output at the end of each encounter of a fly in the time interval. Line 730 revalues the egg load of the fly.

Table 78: The Monte Carlo simulation: 3

```
560   LET m(b) = RND
570   FOR e = 1 to n(2)
580   IF m(b) >= (e-1)/n(2) AND m(b) < e/n(2) THEN LET q(b) = e
590   NEXT e
600   LET s(b) = 1
610   FOR c = 0 TO 8
620   IF i(b) < c THEN LET l(b,c) = 0: IF i(b) < c THEN GOTO 640
630   LET l(b,c) = w(1,c) + f(u+1,i(b)-c, n(2))
640   NEXT c
650   FOR c = 0 to 8
660   IF l(b,c)    =    MAX(l(b,0),l(b,1),l(b,2),l(b,3),l(b,4),l(b,5),l(b,6),
                        l(b,7),l(b,8)) THEN LET r(b) = c
670   NEXT c
```

Table 79: The Monte Carlo simulation: 4

```
680   LET g(q(b)) = g(q(b)) + r(b)
700   IF s(b) = ) THEN LPRINT "clean" b, i(b), g(q(b)), r(b)
710   IF s(b) = 1 AND r(b) = 0 THEN LPRINT "par" b, i(b), g(q(b)), "no"
      r(b)
720   IF s(b) = 1 AND r(b) > 0 THEN LPRINT "par" b, i(b), g(q(b)),"yes"
      r(b)
730   LET i(b) = i(b) - r(b)
760   NEXT b: NEXT u
```